A STUDY OF SERUM PARAOXONASE - 1 ACTIVITY IN PATIENTS WITH METABOLIC SYNDROME

Dissertation Submitted for

M.D DEGREE BRANCH - XIII

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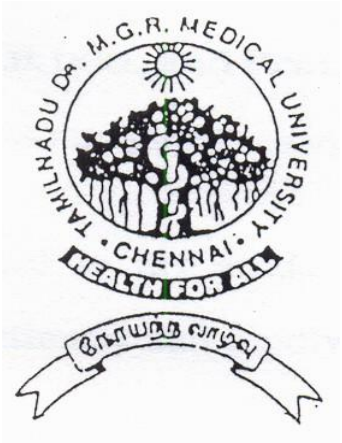

DEPARTMENT OF BIOCHEMISTRY

THANJAVUR MEDICAL COLLEGE,

THANJAVUR

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY,

CHENNAI

APRIL - 2015

CERTIFICATE

This is to certify that dissertation titled "**A STUDY OF SERUM PARAOXONASE–1 ACTIVITY IN PATIENTS WITH METABOLIC SYNDROME"**is a bonafide work done by **Dr.T.RAJALAKSHMI** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2012 to 2015.

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DECLARATION

I, **Dr.T. RAJALAKSHMI** hereby solemnly declare that the dissertation title "**A STUDY OF SERUM PARAOXONASE-1 ACTIVITY IN PATIENTS WITH METABOLIC SYNDROME"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of the Department **Dr.N.Sasivathanam, M.D(Bio).,DGO,** This dissertation is submitted to Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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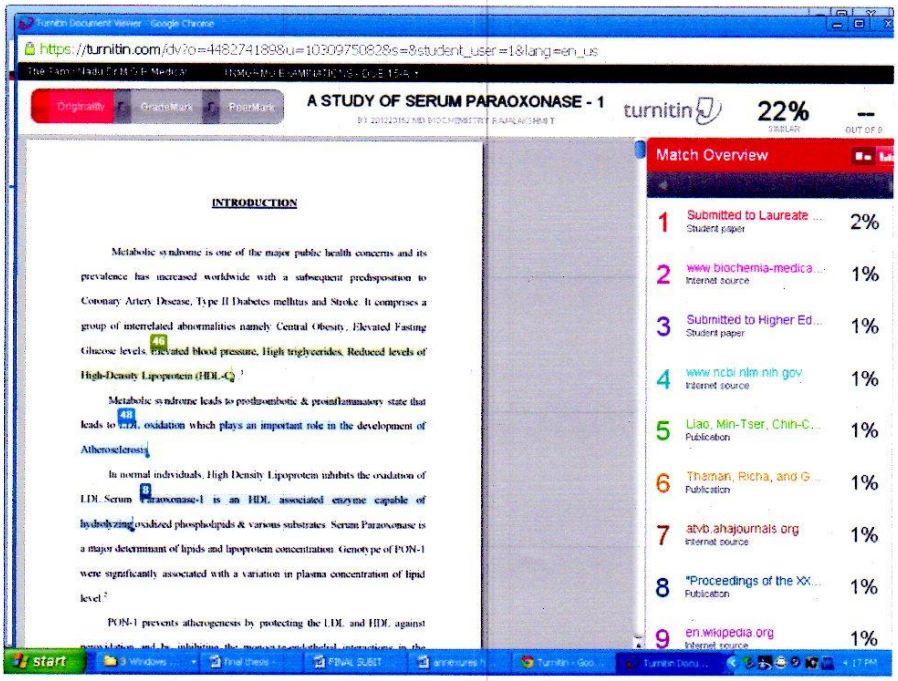

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CONTENTS

ABBREVIATIONS

PON-Paraoxonase

MDA-Malondialdehyde

Mets-Metabolic Syndrome

DM - Diabetes Mellitus

FBG-Fasting Blood Glucose

TGL-Triglycerides

HDL - High density lipoprotein

LDL - Low density lipoprotein

VLDL - Very low density lipoprotein

TNF-α - Tumor necrosis factor α

INF α - Interferon α

IL- 6 - Interleukin 6

PAI - Plasminogen activator inhibitor

CETP - Cholesterol ester transfer protein

LPL - Lipoprotein lipase

PDE 3 - Phosphodiesterase 3

PKA - Protein kinase A

HSL - Hormone sensitive lipase

CVD - Cardiovascular disease

VEGF - Vascular endothelial growth factors

IRS-Insulin Receptor Substrate

PI3 Kinase-Phosphatidyl Inosital 3 Kinase

MAP Kinase-Mitogen activator protein kinase

GLUT-Glucose transporters

FFA-Free Fatty acids

Abstract

Background: Metabolic syndrome leads to prothrombotic & proinflammatory state that result in LDL oxidation which plays an important role in the development of Atherosclerosis. In normal individuals, High Density Lipoprotein inhibits the oxidation of LDL.Serum Paraoxonase-1 is an HDL associated enzyme capable of hydrolyzing oxidized phospholipids & various substrates.Thus, estimation of PON-1 activity in metabolic syndrome is valuable in assessing the risk for atherosclerosis and thereby predicting future cardiovascular complications.

AIMS AND OBJECTIVES

1) To estimate the levels of serum Paraoxonase-1 in patients with metabolic syndrome and to compare the levels with healthy controls.

2) To evaluate the association of Paraoxonase -1 activity & malondialdehyde levels with components of metabolic syndrome.

MATERIALS AND METHODS

PON-1 activity was estimated using the paraoxon (O,O diethyl-O-4 nitro phenyl phosphate) as the substrate for hydrolysis. Fasting Blood glucose was estimated by GOD-POD method. The serum triglycerides were measured by enzymatic (GPO-PAP method).For the determination of total cholesterol, an enzymatic (GPO-PAP) method was used.HDL was measured by Phosphotungstic acid method .VLDL & LDL were calculated by friedwalds

formula.Estimation of Malondialdehyde (MDA) by Thio Barbituric Acid reactivity assay method.

RESULTS

The serum PON1 activity among the Study group (59.32 ±19 U/L) is significantly lower than the control group (154.84 ±30.71 U/L). The Serum Malondialdehyde values in study group (7.81 ± 3.8 µmol/L) is significantly increased, than that of control group (2.809±1.40). As the number of components of Metabolic Syndrome increases mean PON 1 Activity decreases & mean MDA levels increases.

CONCLUSION

This study shows that there is a significant decrease in PON-1 activity in Metabolic Syndrome group and there is significant increase in Malondialdehyde .Since PON-1 is an antiatherogenic and antioxidant enzyme, associated with HDL, reduction in PON-1 activity in Metabolic Syndrome, may play an important role in causation of premature atherosclerosis. Based on the results obtained the present study shows that PON-1 activity may be used as a useful marker for early prediction of atherosclerosis in Metabolic Syndrome.

INTRODUCTION

Metabolic syndrome is one of the major public health concerns and its prevalence has increased worldwide with a subsequent predisposition to Coronary Artery Disease, Type II Diabetes mellitus and Stroke. It comprises a group of interrelated abnormalities namely Central Obesity, Elevated Fasting Glucose levels, Elevated blood pressure, High triglycerides, Reduced levels of High-Density Lipoprotein (HDL-C) .[1]

Metabolic syndrome leads to prothrombotic & proinflammatory state that leads to LDL oxidation which plays an important role in the development of Atherosclerosis.

In normal individuals, High Density Lipoprotein inhibits the oxidation of LDL.Serum Paraoxonase-1 is an HDL associated enzyme capable of hydrolyzing oxidized phospholipids & various substrates. Serum Paraoxonase is a major determinant of lipids and lipoprotein concentration. Genotype of PON-1 were significantly associated with a variation in plasma concentration of lipid level.[2]

PON-1 prevents atherogenesis by protecting the LDL and HDL against peroxidation and by inhibiting the monocyte-endothelial interactions in the inflammatory response to the vascular endothelial cells.[3]

Thus, estimation of PON-1 activity in metabolic syndrome is valuable in assessing the risk for atherosclerosis and thereby predicting future cardiovascular complications.

REVIEW OF LITERATURE

HISTORY OF METABOLIC SYNDROME

In 1947, Dr. Jean Vague, the Marseilles physician, found that abdominal obesity predisposed to atherosclerosis, diabetes and cholelithiasis, but the term "metabolic syndrome" was first used in 1950 and only in late 1970 it came into common practice. [4]

In 1981, Hanefield and Leonhardt used the term ‘‘Metabolic Syndrome’’ to describe the joint incidence of diabetes, hypertension, hyperlipoproteinemia, gout and obesity in combination with an increased incidence of cardiovascular disease, fatty liver and cholelithiasis.[5]

In 1985, Modan proposed that Insulin Resistance or Hyperinsulinemia as a common pathophysiological feature for Obesity, Hypertension and Glucose Intolerance.[6]

In 1988, Gerald M.Reaven, an endocrinologist from Stanford University, interpreted the association of diabetes, obesity, dyslipidemia and arterial hypertension by their pathogenic relationship with the peripheral Insulin-Resistance and he named the above association as “X syndrome”. [7]

The X syndrome was renamed as “The Deadly Quartet” by Kaplan in 1989. It was again renamed as “Insulin Resistance Syndrome” in 1992.

Hjerrman proposed renaming Syndrome X as "Metabolic Cardiovascular Syndrome" or "Atherothrombogenic Syndrome".[8]

The Centers for Disease Control and Prevention approved for a new diagnostic code, ICD-9-CM 277.7 for "Dysmetabolic Syndrome X" in 2001.

Synonyms for Metabolic Syndrome[6]

- Hyperinsulinemia Syndrome /Insulin Resistance Syndrome
- Atherothrombogenic Syndrome
- Metabolic Cardiovascular Syndrome
- Wohlstands syndrome (Germany)
- Dysmetabolic Syndrome X
- Syndrome X plus
- Deadly quartet
- Android Obesity Syndrome
- Syndrome of Affluence
- Plurimetabolic syndrome
- GHO (Glucose intolerance/ Hypertension/Obesity syndrome)

- Syndrome X
- Metabolic syndrome X
- Reaven syndrome
- **HONDA** --- Hypertension, Obesity, Non insulin dependent Diabetes Mellitus,Dyslipidaemia, Atherosclerotic Cardiovascular disease
- **CHOAS** (Australia)- Coronary Artery Disease, Hypertension, Obesity,Atherosclerosis, and Stroke.

DEFINITIONS:

At present, there are four sets of criteria most commonly used for defining the Metabolic Syndrome.

THE METABOLIC SYNDROME-WHO CRITERIA(1998): [10]

- This definition must have the presence of insulin resistance(Type 2 diabetes /IFG/IGT/other evidence of IR like euglycemic clamp) study),along with any two of the following:
- Central obesity : Body mass index $\geq$ 30 kg/m^2or

  Waist: hip ratio> 0.85 for female, > 0.90 for male.
- Dyslipidemia: HDL $\leq$ 0.9 mmol/L for male,

  HDL $\leq$ 1.0 mmol/L for female

  TGL $\geq$ 1.695 mmol/L.
- Blood pressure: $\geq$ 140/90 mmHg.

- Microalbuminuria: Albumin: creatinine ratio ≥ 30 mg/g or urinary albumin excretion ratio ≥ 20 µg/min.

EUROPEAN GROUP FOR THE STUDY OF INSULIN RESISTANCE (EGIR) CRITERIA (1999) [11]

The EGIR felt that Insulin Resistance plays a central role in metabolic syndrome.

- Insulin Resistance is defined as a fasting plasma insulin value greater than 75th percentile.

  Plus any two of the following
- Waist circumference : ≥ 94(male), ≥90 (female)
- Blood pressure: systolic ≥ 140 or diastolic ≥ 90 mmHg.
- TGL ≥177mg/dl.
- HDL: <39mg/dl.

THE NATIONAL CHOLESTEROL EDUCATION PROGRAM (NCEP) ADULT TREATMENT PANEL III (ATP III)-2001:[12]

Metabolic Syndrome is present if ≥3/5 criteria are fulfilled.

- Blood pressure: systolic ≥ 130 or diastolic ≥ 85 mmHg.
- Fasting Triglycerides≥150mg/dl.

- Waist circumference: ≥ 40 inches or ≥ 102 cms for male, ≥ 35 inches or≥ 88 cms for female.
- Fasting plasma glucose: ≥ 110mg/dl.
- Fasting HDL: < 50 mg/dl for female and < 40 mg/dl for male.

The NCEP ATP III definition is most widely used criteria of MetS because of its simple in application & results are readily available.

INTERNATIONAL DIABETES FOUNDATION (IDF)–CRITERIA (2005): [13]

Central obesity and any two of the following:

- Known case of type II Diabetes Mellitus or Elevated Fasting Plasma Glucose (FPG): ≥ 100 mg/dl.
- Fasting Triglycerides ≥ 150mg/dl or on specific medication.
- HDL cholesterol < 50 mg/dl and < 40 mg/dl for women and men respectively.
- Blood pressure >85 mmHg diastolic or>130 mmHg systolic or a known case of hypertension.

Waist circumference with geography – specific values for defining Central obesity.

Central Adiposity by IDFCriteria: [11]

Men	Women	Ethnicity
≥85cm	≥90cm	Japanese.
≥90cm	≥80cm	Central American, Chinese &South Asian.
≥94cm	≥80cm	Middle Eastern, Eastern, Europid& Sub-Saharan African

MODIFIED NCEP ATPIII-CRITERIA FOR THE METABOLIC SYNDROME(2005):[14]

The important changes in this criteria include:

(i)The ethnic difference in central obesity.

i.e., 90 cms in Asian men & 80 cms in Asian women.

(ii) Impaired Fasting Glucose ≥ 100mg/dl

(iii) If patients are receiving drug treatment for individual components, those components are counted as abnormal.

By making these differences the predictive ability to diagnose MS has become highest with modified NCEP-ATP III.

Metabolic Syndrome is present if three or more of the following five criteria are met with

- Waist circumference :

  African Americans, whites, Latin Americans.

  Male- ≥ 40 inches or ≥ 102 cm ,

  Female - ≥ 35 inches or ≥ 88 cm

  Asians-

  Male- ≥ 35 inches or ≥ 90 cm,

  Female - ≥ 32 inches or ≥ 80 cm

- Blood pressure: systolic ≥ 130 or diastolic ≥ 85 mmHg or on treatment.
- Fasting triglyceride (TG) level: Triglycerides≥150mg/dl or on treatment.
- Fasting plasma glucose: ≥ 100mg/dl or on treatment.
- Fasting high-density lipoprotein (HDL) cholesterol level: <50mg/dl (female) and < 40 mg/dl (male) or on treatment.

EPIDEMIOLOGY

- One-quarter of the world's adult population has Metabolic Syndrome.
- In Developed countries like the United States, Metabolic Syndrome affects 47 million Americans.[15]

- 1988-1994, nearly 24% of U.S. adults aged 20 years or older have MetS. , 27% in 1999-2000 and 34% in 2010. [7]
- The prevalence in 7 European countries was approximately 23%.
- It was estimated that 20%–25% of South Asians have developed MS.[17]
- In southern India, the prevalence of MetS was 26.9% in males and 18.4% in females. .[18]
- The incidence of Metabolic syndrome progressively rises with increasing age, reaching a peak between 60 and 69 years and the prevalence increasing from 10% in the 30–39-year age group to 45% in the 60–69-year age group.

RISK FACTORS OF METS[19]

- Obesity
- Diet
- Sedentary life style
- Aging
- Stress
- Smoking
- Disrupted chronobiology / sleep
- Excessive alcohol consumption
- Race.
- History of diabetes

OBESITY

Metabolic Syndrome increases with increasing levels of BMI in men and women.

Two major types of Fat distribution in Metabolic Syndrome

- Apple shaped obesity or Android pattern has more visceral fat and has got increased truncal abdominal fat is more dangerous and it is common among men.
- Gynoid or excess gluteofemoral fat or Pear shaped fat [20]

SEDENTARY LIFE STYLE

- Regular and sustained physical activity positively decreases the individual components of the metabolic syndrome.
- Physical inactivity may be an important modifiable risk factor in the etiology of the metabolic syndrome. [11]

DIET

Total energy intake and diet composition influences insulin sensitivity.

- High mono unsaturated fat improves insulin sensitivity compared with high-saturated-fat diet.If the total fat intake is 38%, beneficial effect of monounsaturated fat is lost.

- High-carbohydrate diet worsens fibrinolysis which leads to increase of PAI-1 in blood.
- Alcohol intake of about >30g/day increases both Bp & TG levels.
- The blood pressure is elevated by increased intake of sodium chloride.[21]

AGEING

- The increase in proinflammatory cytokines is associated with ageing.
- Aging leads to loss of mitochondrial function in various tissues like skeletal muscle leads to generation of Reactive Oxygen Species and oxidative damage of macromolecules. [22]

STRESS

- Stress leads alteration in Autonomic Nervous System and neuroendocrine systems.
- The neuroendocrine responses from the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system stimulate the stress-related cortisol secretion, this contributes to the development of dyslipidemia, abdominal obesity, hypertension and insulin resistance and further leads to CVD [23]

SMOKING

- It reduces insulin sensitivity
- It increases sympathetic stimulation & increases cortisol level.
- It decreases testosterone levels in men which lead to increase in visceral fat.

DISRUPTED CHRONOBIOLOGY/SLEEP

- Disruption in circadian rhythm due to alteration in melatonin levels leads to MetS.
- Healthy individuals restricted to 4 hours of sleep for six consecutive nights causes impaired glucose tolerance and reduced insulin responsiveness following a glucose challenge. [23]

EXCESSIVE ALCOHOL CONSUMPTION

- Protective and detrimental associations have been reported between alcohol consumption and the metabolic syndrome.
- A minimal amount of alcohol consumption has protective effect on prevalence of the metabolic syndrome compared with nondrinking individuals.
- The prevalence of the metabolic syndrome increases with lifetime drinking intensity (total drinks/drinking days over lifetime). [24]

RACE

- Asians and Hispanics have greater risk of metabolic syndrome. [24]

FAMILY HISTORY OF DIABETES

- Family history of type 2 Diabetes mellitus or a history of diabetes mellitus during pregnancy (gestational diabetes) leads to Metabolic Syndrome.

OTHER DISEASES

Polycystic Ovary Syndrome and Nonalcoholic Fatty liver disease that affects a woman's hormones and reproductive system also raises the risk of metabolic syndrome.[25]

ETIOPATHOGENESIS

The most accepted Pathophysiology of the metabolic syndrome is insulin resistance.

Mechanism of Action of insulin

Insulin receptor is a heterotetramer, comprising two α and two β subunits. When Insulin binds to this α subunits, it induces a conformational changes in the receptor, resulting in the activation or phosphorylation of tyrosine kinase, which is present in the intracellular part of β subunits.In addition to phosphorylating itself, insulin receptor catalyzes the tyrosine phosphorylation of various intracellular proteins like Shc, IRS family.[26]

IRS causes activation of PI3K which leads to activation of the 3-phosphoinositide-dependent protein kinase-1 and Akt kinase which leads to metabolic effects of insulin through translocation of GLUT-4 to the cell surface, uptake of Glucose, Synthesis of Glycogen, Lipids & Protein.Akt also activates eNOS in vascular endothelial cells leads to vasodilatation and increased delivery of insulin and glucose to tissues.

Shc protein causes activation of Growth Factor Receptor Bound Protein which leads to MAP kinase pathway activation through SOS, Ras, Raf, Mitogen activated ERK (extracellular regulated kinase) - MEK. This MAP kinase pathway causes endothelin-1 (ET-1) production; expression of

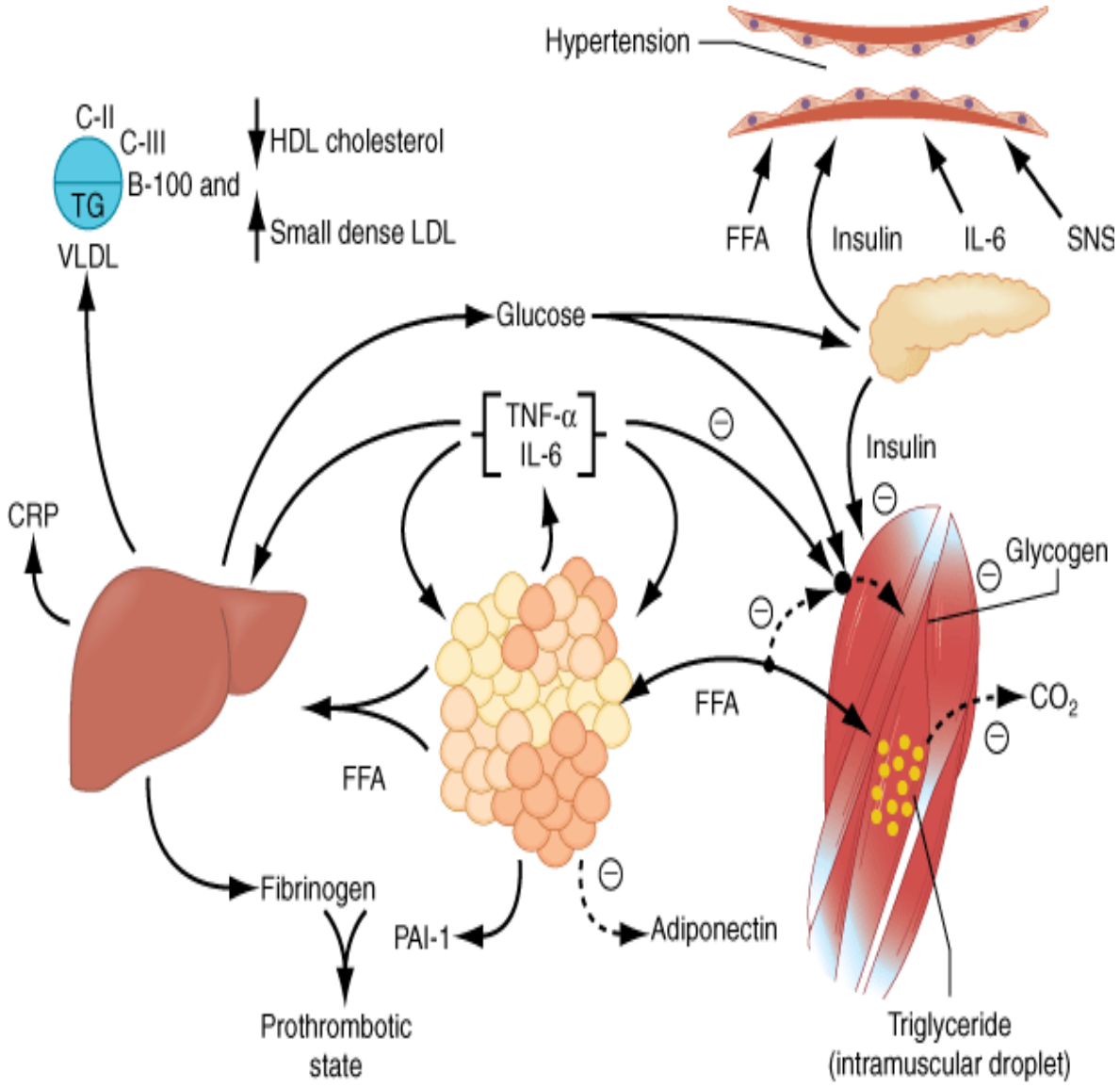
the vascular cell adhesion molecules VCAM-1 and E-selectin, leading to more endothelial-leukocyte interactions, growth and proliferation of vascular smooth muscle cells.[27]

INSULIN RESISTANCE

Insulin resistance is defined as a defect in insulin action which is necessary to maintain euglycaemia or It is defined as a decreased biological response to the normal concentration of circulating insulin.[26]

In Insulin Resistance, MAP kinase pathway is not affected but the PI3K-Akt pathway is affected. Thus, insulin resistance leads to vascular abnormalities which predispose to atherosclerosis.[27]

PATHOPHYSIOLOGY OF METABOLIC SYNDROME



DYSLIPIDEMIA

Excessive release of free fatty acids from adipose tissue into plasma leads to impaired insulin-mediated glucose uptake in muscle. Increased level of circulating glucose increases pancreatic insulin secretion resulting in hyperinsulinemia.

In the liver, FFAs leads to increased synthesis of TGs. FFAs also stabilizes and increases apoB, which leads to increased VLDL production.Metabolism of VLDL causes increased LDL ,which promotes atheroma formation. [28]

The Cholesterol Ester Transport Protein transfers TGL in VLDL to HDL in exchange of cholesteryl esters.The TG-enriched HDL is rapidly cleared by hepatic lipase which leads to decreased plasma HDL level. [11]

In skeletal muscle, sudden raise in FFA leads to increased DAG and long chain fatty acyl COA formation causes activation of protein kinase, which phosphorylates serine/threonine sites of insulin receptor substrate-1, leading to inability of insulin to activate downstream events.[27]

The defective mitochondrial function and excessive intracellular accumulation of fatty acids leads to increase in the production of Reactive Oxygen Species, causes activation of the proinflammatory Nuclear Factor Kappa B pathway, thereby increasing insulin resistance.[29]

CENTRAL OBESITY

Central obesity is more metabolically active then peripheral fat. Waist circumference has to be routinely measured to assess individuals for increased risk for Insulin Resistance related CardioVascular Disease.

Visceral fat releases their metabolic products directly into portal circulation, which carries blood straight to the liver.[30] Therefore free fatty acids are poured into the liver. Free fatty acids also accumulate in the pancreas, heart and other organs. This leads to organ dysfunction, producing impaired regulation of insulin, blood sugar and cholesterol as well as abnormal heart functions. This is known as Lipotoxicity.

Adipose tissue produces several inflammatory cytokines (adipokines) like Tumor Necrosis Factor α and interleukin-6 and these are proinflammatory and contribute to insulin resistance and vascular dysfunction.[31] But, Adiponectin is a protective adipokine that increases insulin sensitivity in the liver, skeletal muscle glucose uptake and fatty acid oxidation, decreases hepatic glucose production. [30]

HYPERTENSION

Insulin causes vasodilatation, so, in insulin resistance vasodilatory effect is lost but sodium resorption function is preserved.[11]Fatty acids themselves can cause vasoconstriction.

Hyperinsulinaemia leads to increased sympathetic nervous system activity and thus contribute to the development of hypertension.

PROINFLAMMATORY STATE

Adipocyte-generated inflammatory cytokines correlates well with insulin resistance. The Circulating signal molecules from fat includes FFAs, Adiponectin, IL-6 (IL-6 increases CRP production particulary in liver), Resistin, Leptin and TNF-α.[31]In Metabolic Syndrome, the inflammatory cytokines causes Insulin Resistance.

PROTHROMBOTIC STATE

Increased plasma Plasminogen Activator Inhibitor -1, Thrombin-Activatable Fibrinolysis Inhibitor-1 and Fibrinogen are associated with metabolic syndrome.[32] Fibrinogen and CRP , rises in response to a high-cytokine state. Thus, proinflammatory and prothrombotic states are metabolically interconnected.

Triglycerides have proatherogenic effects by promoting a procoagulant state due to enhanced Factor VII activity [33]

TPA converts plasminogen to plasmin which acts on fibrin causing clot dissolution; this is inhibited by plasminogen activator inhibitor.PAI increases with increase in BMI, LDL, and Systolic & Diastolic BP. [33]

COMPLICATIONS OF METABOLIC SYNDROME

CARDIOVASCULAR DISEASE

The mechanisms of cardiac dysfunction in the MetS are lipid accumulation, increased fibrosis and stiffness, altered substrate utilization, abnormal autophagy, altered calcium homeostasis, mitochondrial dysfunction increased oxidative stress, increased fatty acid oxidation and enhanced NADPH oxidase activity.[34]

The prothrombotic and proinflammatory states in Metabolic Syndrome lead to cardiovascular disease.[35]

TYPE 2 DIABETES MELLITUS

Increased NEFA leads to increased utilization of NEFA as energy source for muscle leads to increased acetyl CoA & citrate which inhibit PFK1 leads to decreased glucose metabolism & Defects in insulin action leads to impaired glucose tolerance & Type 2 diabetes.[36]

NON ALCOHOLIC FATTY LIVER DISEASE

- Increased FFA acid levels in Metabolic Syndrome leads to increased esterification of FFA &increased triglyceride synthesis & accumulation of triglycerides leads to steatohepatitis.

- In Mets, Non- alcoholic fatty liver disease (NAFLD) is more common which leads to non-alcoholic steatohepatitis (NASH), the inflammatory form of liver steatosis.
- As the prevalence of overweight/obesity and the metabolic syndrome increases, NASH may become one of the common causes of end-stage liver disease and hepatocellular carcinoma.[11]

HYPER URICEMIA

- Uric acid production is linked to glycolysis which is controlled by insulin.
- Insulin inhibits the activity of glyceraldehyde-3-phosphate dehydrogenase,leads to conversion of glyceraldehyde-3-phosphate to Ribose-5-phosphate which in turn converts to Phospho ribosyl pyro phosphate leads to increased uric acid production.[37]

CHRONIC KIDNEY DISEASE

IR promotes kidney disease by mechanisms such as

- Activation of the sympathetic nervous system, sodium retention, decreased Na+, K+-ATPase activity, and increased GFR.
- Endoplasmic reticulum (ER) stress seems to be the factor-linking inflammation and IR at the molecular level.

- The suppression of insulin signalling via phosphorylation of the insulin receptor substrate (IRS-1) due to activation of c-Jun N-terminal kinase (JNK) plays an important role .
- Renal ER stress is associated with alteration of nephrin N-glycosylation in podocytes, which is the underlying factor in the pathogenesis of proteinuria, leads to chronic kidney injury with tubulointerstitial damage.
- IR and inflammatory cytokine release leads to basement membrane thickening, glomerular mesangial expansion, podocytopathy, and the loss of slit pore diaphragm integrity leading to glomerulosclerosis and tubulointerstitial injury. [38]
- Metabolically overloaded FFA in PTECs causes increased expression of proinflammatory cytokines, such as TNFα & MCP1 and, and lead to epithelial-mesenchymal transition.
- High triglyceride levels is as such a risk factor for proteinuria development.

Hypertension and hyperinsulinemia are individual risk factors for MicroAlbuminuria and subsequent development of Chronic Kidney Disease.Hyperinsulinemia itself is a risk factor for chronic kidney disease.

POLYCYSTIC OVARY SYNDROME

- Hyperinsulinemia increases IGFs in the liver and increases the production of androgens in the ovaries.
- The direct effect of insulin and IGF-1 causes increased 17-hydroxylase activity in the ovaries, causing an excessive production of androgens, particularly Testosterone and its precursor, 17-hydroxyprogesterone (17-OHP).

- IGF-1 prevents the conversion of Testosterone into Estrogens by inhibiting the enzyme Aromatase.
- Insulin potentializes the action of LH in the ovaries.
- Hyperinsulinemia decreases the hepatic production of SHBG, the protein that carries the sex hormone, and of IGFBP-1, the protein that carries IGF-1, thus contributing to a broader action of free testosterone (FT) and IGF-1, respectively in target-cells.[39]
- The inappropriate secretion of gonadotropins is associated to the PCOS. The increased production of gonadotropins is related to increased activity of GnRH pulse generator and to pituitary response to GnRH leads to increased LH secretion than FSH.
- Increased LH leads to increased stimulation of stroma & theca cells to produce increase ovarian androgen secretion.

- Decreased FSH leads to decreased follicular maturation which leads to chronic anovulation [40]
- PCOS is highly associated with the metabolic syndrome, with prevalence between 40 and 50%. Women with PCOS are 2–4 times more likely to have the metabolic syndrome than are women without PCOS.

OBSTRUCTIVE SLEEP APNOEA (OSA)

In metabolic syndrome ,there is altered hypothalamo-pitutary adrenal axis ,decreased Ghrelin,decreased Adiponectin & increased Leptin with Leptin resistance all these factors leads to OSA.

OSA is commonly associated with Insulin Resistance, Obesity, Hypertension, increased circulating Cytokines, IGT.[41]

RELATIONSHIP BETWEEN METABOLIC SYNDROME, OXIDATIVE STRESS, INFLAMMATION, ATHEROSCLEROSIS &CARDIO VASCULAR DISEASE.

The metabolic syndrome is associated with a higher fraction of oxidized LDL which exerts several biological effects which contribute to the initiation & progression of the atherosclerotic process.[42]

MetS increases TNF-α which activates and induces expression of specific adhesion molecules like VCAM, ICAM, and E-SELECTIN etc on

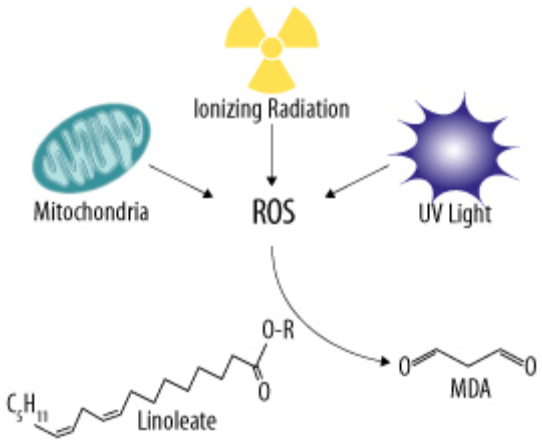
the endothelial cells. It also influences the behavior of circulating monocytes, increasing its adhesion to the endothelium. Monocytes adhere and penetrate in response to chemotactic factors like MCP-1 which is up regulated in MetS. The extracellular lipid begins to accumulate in intima which is often associated with proteoglycans of extra cellular matrix. Sequestration within the intima separates lipoproteins from plasma antioxidants and favours oxidative modification.[43,44]

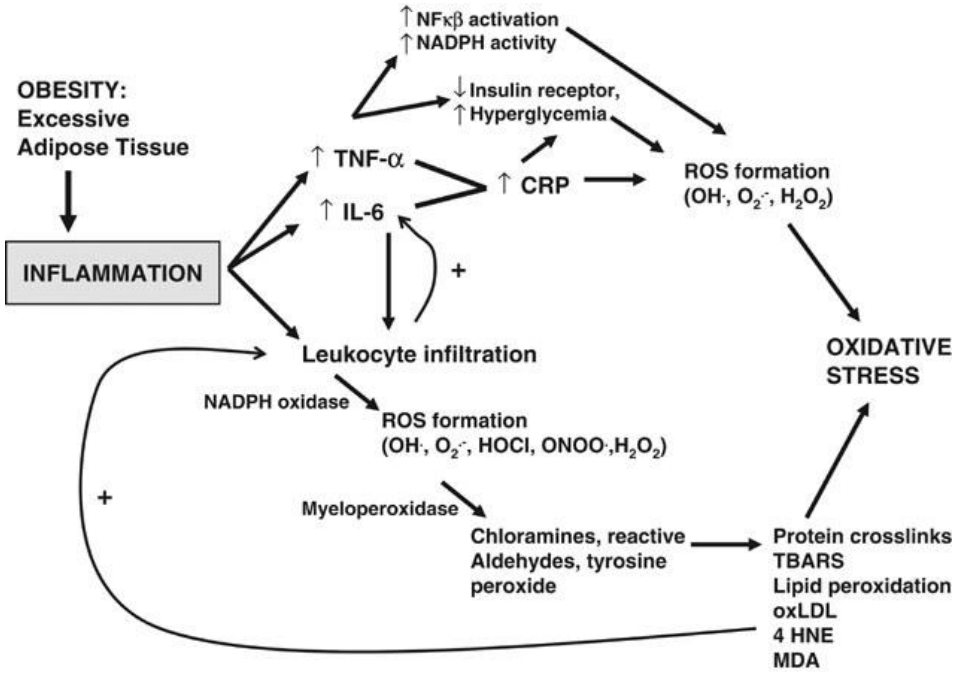
The accumulation of oxidized LDL, which activates the subsets of smooth muscle cells and macrophages leads to gelatinase production and upstream localization of a vulnerable plaque phenotype. [33]

Dyslipidemia in metabolic syndrome (↓ HDL & ↑ TGL) reduces reverse cholesterol transport and increases the formation of small dense LDL, which is more prone for oxidation. Small dense LDL enters the arterial intima more easily and binds more readily to the proteoglycans, through specific sequence of apoB, than the larger fraction. Binding of LDL in the arterial intima increases the dwelling time, and provides opportunity for oxidation of LDL lipids.[42]

The LDL oxidation is a free radical driven chain reaction that is initiated by the free radical, attacking the double bond associated with PUFA[30].This process starts when a hydrogen atom is removed from a methylene group (-CH2-)in an unsaturated fatty acid by the free radical(OH•). This leaves a fatty acid as a radical itself, and the most likely outcome of this is that, it will react with molecular oxygen in the cell membrane to form a peroxyl radical.[34]

MECHANISM OF LIPID PEROXIDE FORMATION





Radical damage to unsaturated fatty acids in cell membranes& plasma lipoproteins lead to the formation of lipid peroxides, then highly reactive dialdehydes that can modify protein& nucleic acid bases. The dialdehydes formed from lipid peroxides can be measured by reaction with thiobarbituric acid.[45]

$R\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{–}CH_2\text{-}COOH$ **(FATTY ACID)** + $OH^{\bullet} \rightarrow$

$R\text{-}C^{\bullet}H\text{-}CH{=}CH\text{-}CH_3\text{-}CH_2\text{-}CH_2\text{-}COOH$**(FATTYACID RADICAL)**+ H_2O

$R\text{-}C^{\bullet}H\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}COOH + O_2 \rightarrow$

FATTY ACID

$R\text{-}C(OO^{\bullet})H\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}COOH$

PEROXYLRADICAL

$R\text{-}C(OO^{\bullet})H\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}COOH + R\text{-}CH_2\text{-}CH{=}CH\text{-}CH_2\text{–}CH_2\text{-}COOH$

PEROXYL RADICAL ↓ **FATTY ACID**

$R\text{-}C(OOH)\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}COOH + R\text{-}C^{\bullet}H\text{-}CH{=}CH\text{-}CH_2\text{-}CH_2\text{-}COOH$

LIPID PEROXIDE **FATTY ACID RADICAL**

This radical can then react with another fatty acid resulting in formation of lipid peroxide molecule (lipid .OOH) and fatty acid radical.[46] This chain of reaction results in generation of intermediate compounds like malondialdehyde and 4 hydroxynonenol.[47] These compounds then bind to apo B100, of LDL giving it an increased negative net charge, and

rendering it unrecognizable by native LDL receptors but facilitate recognition and uptake by scavenger receptors. RBC's from disrupted microvessels causes deposition of heme in the extracellular space ,acts as a source of iron which catalyse the oxidation reaction by Fenton reaction .[48]

$$\text{Lipid-OOH} + Fe^{2+} \rightarrow \text{Lipid } O^{\bullet} + OH^{-} + Fe^{3+}$$

Fe2+ reacts with the lipid peroxides by splitting the oxygen-oxygen (o-o)bond to form a hydroxide ion and an alkoxyl radical (R-O•)

Monocyte derived macrophages, endocytose OxLDL via multiple receptors, including both class A and class B scavenger receptors (the expression of which is increased in Mets), lectin like oxidized LDL receptor -1 and lipoprotein lipase .Uptake of lipid in excess leads to formation of macrophage foam cells laden in cholesterol esters. In addition foam cells can develop similarly from vascular smooth muscle cells by uptake of OxLDL via classA and classB scavenger receptors .[49]

Oxidised LDL has several pro atherogenic properties including, the rapid uptake by macropages to form foam cells, chemoattraction for circulating monocytes, promotion of the differentiation of circulating monocytes into tissue macrophages, and inhibition of the mobility of resident macrophages. It is also cytotoxic to several types of cells and immunogenic.[50]

Macrophage foam cells, leucocytes and resident vascular wall cells can secrete inflammatory cytokines and growth factors (TNFα,IL-1,MCP-1, PDGF,FGF,TGF,etc) that amplify leucocyte recruitment and causes smooth muscle cell migration and proliferation. Thus conversion of fatty streak to fibrous plaque occurs by accumulating vascular smooth muscle cells and accumulating a complex extracellular matrix that consists of proteoglycans, collagen and elastin.[51]

MALONDIALDEHYDE

- Malondialdehyde is an organic compound with the molecular formula $CH_2(CHO)_2$.
- Malondialdehyde mainly exists in the enol form

  $CH_2(CHO)_2 \rightarrow HOCH=CH-CHO$

- In water, the trans-isomer predominates, but in organic solvents, the cis-isomer is present.
- Malondialdehyde is a highly reactive compound.
- It is not typically observed in pure form.
- Malondialdehyde is generated from reactive oxygen species (ROS), and as such is assayed in vivo as a bio-marker of oxidative stress
- Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde.[34]

- It is a reactive electrophile species which causes toxic stress in the cells
- It forms covalent protein adducts known as advanced lipoxidation end-products.
- Malondialdehyde forms DNA adducts by reacting with deoxyadenosine and deoxyguanosine in DNA, the primary one M_1G is mutagenic. The arginine residues in guanidine condenses with malondialdehyde to give 2-aminopyrimidines.[52]
- Human aldehyde dehydrogenase1A1 is capable of oxidizing malondialdehyde.

MALONDIALDEHYDE IN METABOLIC SYNDROME

Malondialdehyde can be formed during lipid peroxidation of PUFA, by the action of human platelet thromboxane synthetase on prostaglandins PGH2, PGH3, PGG2, and by action of polyamine oxidase and amine oxidase on spermine. Increased triglycerides &LDL-c, decreased HDL are the effective factors in the development of oxidative damage in metabolic syndrome.[53]

* Hypertrophic adipocytes secrete cytokines (IL-6, TNF-α) and monocyte chemoattractants (MCP-1) and are characterized by macrophage infiltration generating global proinflammatory profile.

Additionally, macrophage activation leads to NADPH oxidase overexpression and activation, implicated in ROS production. These ROS can oxidize the cell membrane lipids breaking their molecules with consequent increase in their plasma by-products.

* Visceral ectopic fat deposition coexists with hypertriglyceridemia promoting intracellular lipotoxicity, especially in hepatocytes and muscle cells . In hepatocytes, increased fatty acids supply does not essentially result in activation of β-oxidation. Hepatocyte accumulation of esterified fatty acids constitutes a stressful stimulus that result in mitochondrial dysfunction with increased ROS production.
* LDL is the lipoprotein most vulnerable to oxidation so, in metabolic syndrome there is increased oxidative stress.
* Antioxidant activity of HDL activity also decreased in metabolic syndrome.
* Hyperglycemia-induced oxidative stress is characterized by the presence of advanced glycation end-products (AGEs) . AGEs can oxidize lipids in cell membranes leading them to instability and consequent degradation to LPO by-products [54]
* Hyperglycemia in metabolic syndrome is the main source of free radicals.Glucose in its enediol form in a transition metal dependent

reaction oxidized to enediol radical anion that is converted into ketoaldehyde & superoxide radical .

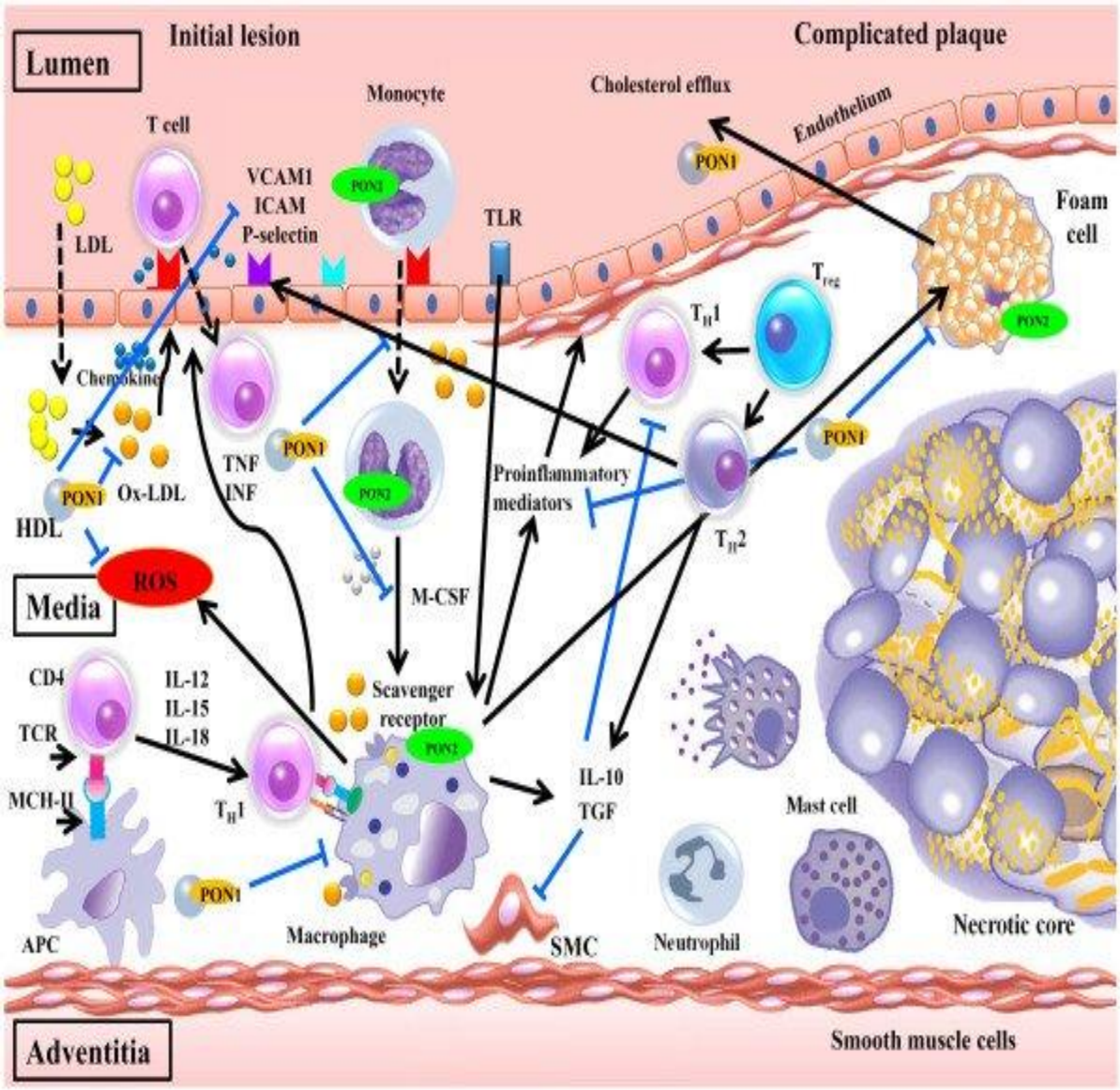
* Hyperglycemia also promotes lipid peroxidation of LDL by superoxide dependent pathway resulting in the generation of free radicals.[55]
* Nitric oxide helps in maintenance of vascular homeostasis. It also decreases platelet and leukocyte adhesion to endothelial cells and regulates vascular tone .Patients with hypertension have low levels of NO leads to increased oxidative stress and free radical formation, augmented platelet adhesion and aggregation, and a change in the arachidonic acid cascade metabolism, all these leading to the acceleration of the atherosclerotic process.[56]
* In Metabolic syndrome there is increased generation of inflammatory cytokines which also leads to generation of free radicals

All these mechanisms lead to increased oxidative stress & increased generation of free radicals & malondialdehyde in metabolic syndrome.

ATTENUATION OF ATHEROSCLEROSIS:

LDL can be protected from oxidation by antioxidants, which can act directly on the LDL and indirectly on the cellular oxidative machinery, and by HDL associated enzyme, Paraoxonase which converts OxLDL to a non atherogenic particle. The ability of HDL to inhibit oxidation of LDL and promote macrophage cholesterol efflux is through the action of several of its proteins, particularly Paraoxonase – 1.[57]

MECHANISM FOR ATHEROSCLEROSIS DEVELOPMENT AND THE ROLES OF PON1 AND PON2 AGAINST ATHEROSCLEROSIS.



PON-1 IN METABOLIC SYNDROME:

PON-1 is the component of HDL, which is responsible for the antiatherogenic properties of HDL which can protect against LDL oxidation [58]

- In Mets, there is increase in number& size of adipose tissue cells .When engorged with fat; adipocytes release leptin, resistin, cytokines & TNF-α.
- TNF-α inhibit LCAT expression & activity which leads to alteration in subfraction of HDL thus it leads to decrease in Paroxonase activity.
- TNF-α decreases ATP binding cassette protein & decreases ABC-G1&A1 expression.
- TNF-α also decreases apo-A1& apo-AIV expression all these leads to decline in HDL levels which is associated with decline in Paraoxonase activity
- Leptin exerts atherogenic property & generates free radicals.
- Leptin directly inhibits paraoxonase levels.
- Increase in Leptin leads to binding of leptin hydrophobic peptide to HDL & inhibit binding of paraoxonase to HDL.
- Leptin increases serum Amyloid -A protein which leads to replacement of ApoA1 in HDL..

- Leptin enhances oxidative stress through generation of ROS & stimulates the secretion of inflammatory cytokines& other acute phase proteins.[59]
- This Inflammatory cytokines and acute phase proteins causes reduced PON-1 enzyme activity.
- Apo A1 & Clustrein are necessary for Paroxonase-1 stability & its optimal activity.[59]
- Paraoxonase is a lipid dependent enzyme. Conformation of paraoxonase within hydrophobic environment is essential for its activity.
- Phospholipids especially those with long chain fatty acid stabilize PON enzyme& require for binding of PON at the lipoprotein surface.
- Lipid composition or products of lipid peroxidation modulate the structural organization & physio chemical properties of lipoproteins. It also alters percentage content of TG/protein & Cholesterol/protein which affects PON1 activity.

Lipid peroxidation products, Oxidized LDL, oxidized palmitoyl or arachidonyl phosphatidyl choline& oxidized cholesterol arachidonate act as inactivators of enzyme activity.[60]

PARAOXONASE:

* Paraoxonase (PON) is an aryl dialkyl phosphatase (E.C 3.1.8.1). It is a multifunctional antioxidant enzyme, which can protect against LDL oxidation.[57]
* It is a calcium dependant esterase that is known to catalyze hydrolysis of various substrates including organophosphates and aryl esters like phenyl acetate and carbamates.[61]
* Its has a ability to hydrolyze paraoxon, a metabolite of insecticide parathion.[62]
* Three forms of Paraoxonase enzymes are synthesised by liver and are 60-65% similar to each other at amino acid level[63].
* PON gene family consists of three related genes in the order of PON-1, PON-3 and PON-2, located on long arm of chromosome 7Q 21.3 with 70% identity in nucleotide sequences.[64]
* PON- 1, PON-2 and PON-3 have 9 exons.
* PON-2 gene is the oldest member of gene family.
* PON-1 mRNA is primarily expressed in liver and PON-3 mRNA primarily in liver and also in kidney.
* PON-2 mRNA is expressed in tissues like kidneys, liver, lungs, small intestine, placenta, spleen, stomach, testes and cells of arterial wall.

* PON-1 and PON-2 have similar function in human.
* PON-1 and PON-3 reside on circulating HDL-cholesterol but PON-2 is not associated with lipoproteins.[65]

PARAOXONASE 1:

* PON-1 is a protein of 354 amino acids with molecular mass of 45 kDa and it is synthesised mainly by the liver.[65]
* PON-1 in serum is exclusively located on HDL in human, which is responsible for the antiatherogenic properties of HDL and prevents LDL oxidation.[66]
* PON-1 can bind reversibly with organophosphates and hydrolyze it and thus form main means of protection of nervous system against the neurotoxicity of organophosphates.[67]
* PON-1 increases the hydrolytic breakdown of lipid hydroperoxides formed on phosphatidyl choline and cholesteryl esters, and also protects HDL from oxidation and increases its ability to induce macrophage cholesterol efflux.[68]
* PON-1 has 2 calcium binding sites, one important for catalytic activity, while other one for stability of enzyme.
* Presence of calcium is required for enzymatic activity and the usage of EDTA destroys PON-1's activity and stability[69]
* It is shown that many amino acid residues of

- Glutamine, Tryptophan, Aspartate, Histidine, Tryptophan are important for organophosphates and aryl esterase activities.

* In addition, important amino acid residues of PON-1 are 3 cysteine residues at position 42, 284 and 353.
* C_{284} – is free while C_{42} and C_{353} form disulphide bonds.
* C_{42} and C_{353} are important for secretion and catalytic activity of PON 1. Exchange of C_{42} and C_{353} with alanine results in inactivation and decrease in secretion of enzyme.
* It is assumed that C_{284} located in close proximity to the active center of the enzyme is responsible for orientation or binding of substrate.[65]

LOCATION:

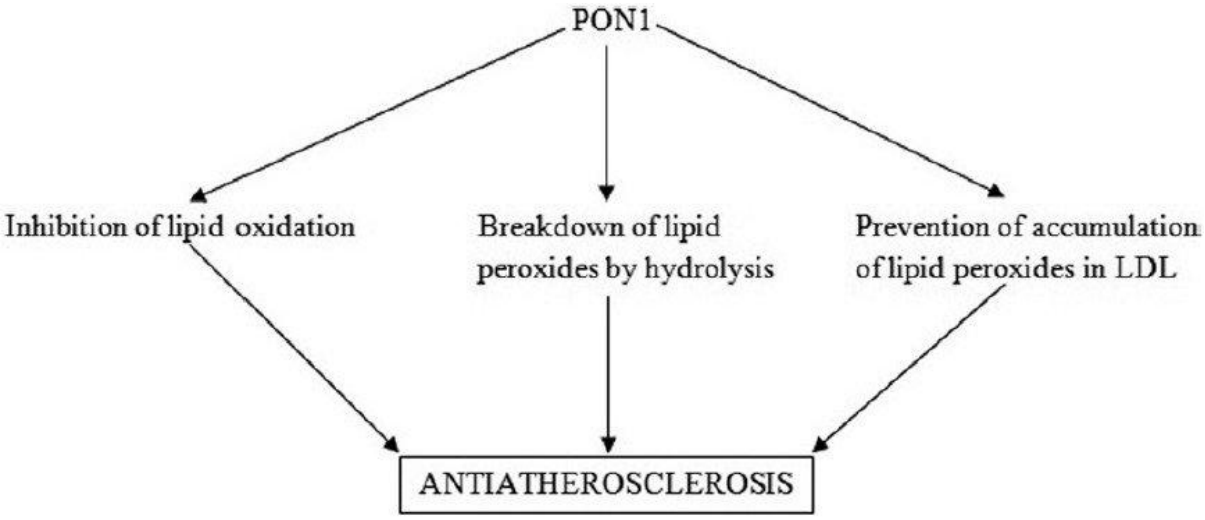
* PON-1 is located on a subfraction of HDL that contain APO A_1 and Clusterin (APO-J).[71]
* In addition to HDL, PON-1 is associated with Tgl rich lipoproteins like postprandial VLDL and chylomicrons but not with LDL.[72]

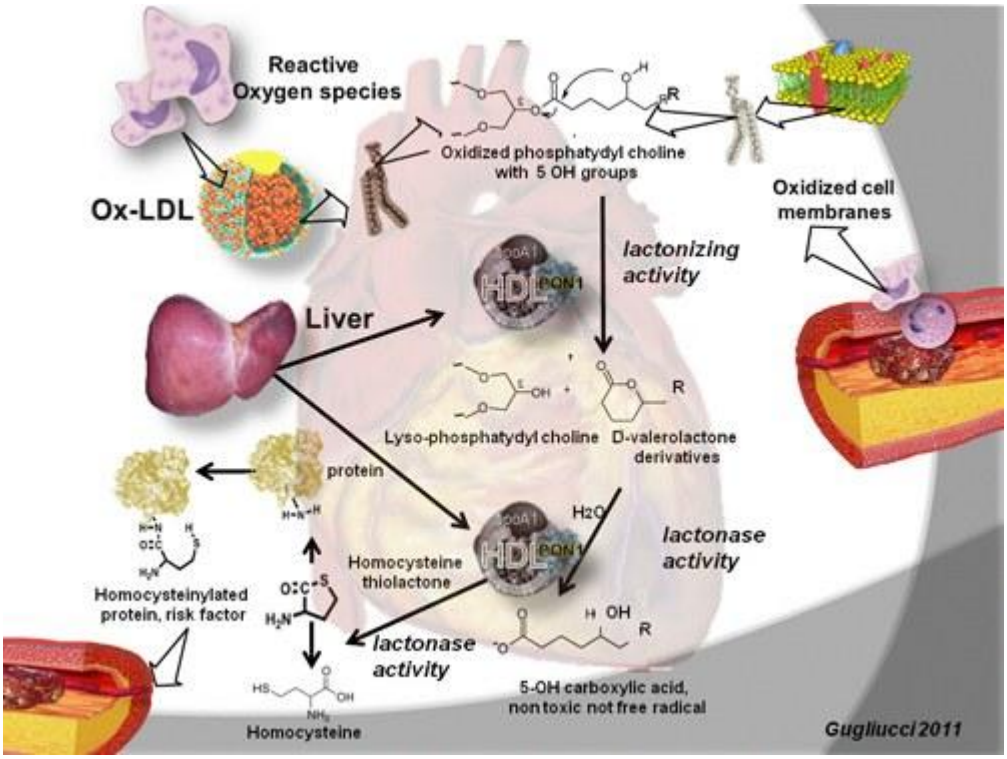
STRUCTURE:

PON-1 contains six bladed beta propeller and each blade has 4 strands. In the central tunnel of PON propeller there are two calcium ions which are 7.4A˚apart.The calcium ion in the central section is stabilizing

this structure, because its dissociation leads to irreversible denaturation of protein. The calcium ion in the upper section is catalytic and its removal results in inactivation of PON towards paraoxon and phenyl acetate. The unique active site lid is also involved in HDL binding.[69, 74, 75] Mature protein of PON-1 retains its hydrophobic signal sequence on the N-terminal region from which, methionine residue is removed. The retained N terminal signal peptide is essential for the association of PON-1 with HDL.

FUNCTIONS OF PON-1





FUNCTIONS OF PON-1:

- PON-1 possess lactonase, arylesterase and organophosphatase activities.[76]
- PON-1 hydrolyzes oxons of insecticides like chlorpyrifos, diazinon and parathion.
- PON-1 also hydrolyzes nerve agents like Soman and Sarin.[77]
- PON-1 hydrolyzes esters like phenyl acetate, thiophenylacetate and 2-naphthyl acetate
- PON-1 also hydrolyzes aromatic and aliphatic lactones as well as cyclic carbonates like dihydrocoumarin, homogentisic acid lactone,butyrolactone.[73]
- It also catalyzes the reverse reaction, lactonisation of γ and δ hydroxy carboxylic acids.[65]
- PON-1 metabolises lactone and cyclic carbonate containing drugs like Prolifloxacin, Spironolactone, Lovastatin, Simvastatin and Mevastatin. It is also responsible for Pilocarpine hydrolysis.[78,79]
- It also has low levels phospholipase-A_2 and peroxidase activity.[80]
- PON-1 possesses antiatherogenic activity. It protects both HDL and LDL from oxidation by its ability to hydrolyze specific oxidised phospholipids and cholesteryl linoleate hydroperoxides in oxidised LDL, and also decreases the macrophage uptake of OX- LDL.[81]

- PON-1 reduces the OX-LDL induced MCP-1 production by endothelial cells.[77]
- It also protects proteins against N-homocysteinylation by hydrolyzing homocysteine thiolactone.[82,83]
- It degrades bioactive phospholipids such as platelet activating factor thereby prevents intravascular coagulation.[80]
- PON metabolizes 5-OH eicosatetraenoic acid and 1,5 lactone and 4-OH docosahexaenoic acid which are the products of both enzymatic and non-enzymatic oxidation of arachidonic acid and docosahexaenoic acid respectively and may represent PON's endogenous substrates.[80]

PARAOXONASE-2:

PON-2 is expressed in many tissues like liver, lungs, testis, placenta, heart and arterial wall cells.[84]

- It is an intracellular protein found in endoplasmic reticulum and nuclear membrane.[84]
- The molecular mass is approximately 44kDa.[84]
- It is secreted from the cell only in small amounts and the enzyme may be rapidly degraded following secretion.[62]
- It is not associated with lipoproteins.

- It has got antioxidant properties and it decreases the oxidation of LDL.It also able to reverse the oxidation of minimally modified LDL (mm LDL).
- It lacks paraoxon and phenyl acetate hydrolyzing activity.[65]

PARAOXONASE-3:

- PON-3 is discovered at the last. It is synthesised in the liver and is present along with HDL in serum, but in much minute levels than PON-1.
- it is also expressed in kidneys.[84]
- The molecular mass is 40 kDa.
- It has got antioxidant properties and it prevents formation of minimally modified LDL and inhibits minimally modified LDL induced monocyte chemotactic activity.
- PON-3 is similar to PON-1 but differs from its substrate specificity. PON-3 lacks paraoxon and phenyl acetate hydrolysing activity and is not regulated by Oxidised lipids and by inflammatory changes.[65]
- Of the three, PON-1 is the most investigated and best understood member of PON family.

PON AND HDL:

- PON-1 is an important component of HDL responsible for the ability of HDL to prevent LDL peroxidation.[85]
- PON-1 is located on a subfraction of HDL that contains APO A_1 and clusterin (APO-J).[86]
- PON-1 activity is highest in HDL-3 and APO-J containing HDL[87].
- Association of PON-1 with HDL is necessary for maintaining the normal serum activity.
- PON-1 is anchored to the HDL lipids by its hydrophobic N-terminal end, and also to be bound to APO-$A_{1.}$
- The N- terminal hydrophobic signal peptide is the structural requirement for binding of PON-1 to HDL and HDL provides the optimal physiological acceptor complex which stimulates secretion and stabilizes the secreted enzyme.[73]
- The conformation of enzyme within the hydrophobic environment of HDL is crucial for its activity.[88]
- HDL is bound on the cell membrane via scavenger receptor B_1 (SRB1). PON-1 which was inserted in to the external surface of the cell membrane is then transferred on HDL during transient association of the lipoprotein with the cell.[89]

- Serum PON-1 activity and concentration are correlated with HDL-cholesterol and APO A_1 concentration in most healthy population but the relationship is not a strong one, as the PON-1 containing HDL being a subspecies, the concentration of which can vary considerably, independently of the major part of HDL.[85]
- Changes in ratio of HDL subfraction may alter the stability and antioxidant capacity of PON-1.[89]

ANTI ATHEROGENIC ROLE OF HDL:

Traditionally, the role of HDL in reverse cholesterol transport has been involved to explain its anti atherosclerotic action. Small HDL precursors known as pre β HDL, in the tissue fluid take free cholesterol from the cell membranes. Once on the pre β particle, the free cholesterol is esterified by the action of LCAT (Lecithin cholesterol acyl transferase) which renders it more hydrophobic. The cholesterol ester moves to the centre of the particle and gradient for the movement of free cholesterol from the cell membrane to the pre β HDL is established. Continued uptake of cholesterol results in formation of larger α migrating HDL3, which diffuse from the tissue fluid in to the plasma. Once in the circulation, HDL remodelling occurs through the action of CETP (cholesterol ester transfer protein), PLTP (phospholipid transfer protein), hepatic and lipoprotein

lipase and the transfer of apolipoproteins from other lipoproteins, resulting in mixture of HDL2, and HDL3, which fall into two main categories.

1) Those containing apoA1 but no apoA2

2) Those containing both.

The fate of cholesterol within the HDL pool, can be taken up by the liver for reuse or it can be transferred back to VLDL or LDL by CETP.[90]

TWO THEORIES OF ANTI OXIDATIVE ACTION OF HDL

Two overlapping theories are 1) Direct Metabolism and 2) Transfer theories.

In Direct Metabolism theory, HDL comes in contact with LDL in the sub intimal space and acts to prevent LDL oxidation, by hydrolyzing lipid hydroperoxides, with the help of PON-1.It is shown that in the absence of PON- 1, HDL is unable to destroy the biologically active lipids in oxidized LDL.

In Transfer theory, HDL acted to protect LDL against lipid peroxidation, by acting as a reservoir for lipid peroxides generated on LDL and therefore breaking the chain of lipid peroxide propagation. Once attached to HDL, phospholipid hydroperoxides (PLHP) could be hydrolysed by HDL associated PON-1, Platelet activating factor acetyl hydrolase(PAF-AH),and LCAT either singly, or in combination to give

non –atherogenic products, or could be transferred to cholesterol by LCAT to form oxidized cholesteryl esters in HDL.HDL then transfers the oxidized cholesteryl esters to the liver for disposal.[63,64,65] Thus the protective effect of HDL may not be dependant on the absolute levels of HDL cholesterol in the blood, but rather the abundance of HDL particles which contains the protective enzymes PON-1and PAF-AH.[80]

REGULATION OF PON ACTIVITY:

GENETIC FACTORS:

The serum level of PON 1 in an individual is relatively stable over time; where as the enzymatic activity of PON 1 varies among individuals by 10- 40 folds. The inter individual variability is due to amino acid polymorphism.[93]

PON 1 has 2 amino acid polymorphism, one at position-55(methionine/ leucine) PON-1 L55M, other at position 192(arginine / glutamine) PON-1 Q192R. Paraoxon hydrolytic activity is greatest with PON-1-192RR and PON-55 LL individuals, and least with PON- 1 192 QQ and PON 1 55MM. Heterozygotes have intermediate levels of activity. On the other hand, the capacity of PON alloenzymes to protect LDL from oxidation is the complete reversal of paraoxon hydrolytic activity. The PON1 55MM / PON1 192 QQ is associated with greatest protective

capacity. These alloenzymes are also most active in hydrolyzing diazoxon and nerve gases Sarin and Soman.[72,94]

All alloenzymes have similar hydrolytic activity on phenylacetate, 2naphthylacetate, chlorpyrifos oxon etc. Glu 192 Arg polymorphism results in decrease in serum PON activity possibly caused by decreased affinity of the Arg 192 polymorphism to the HDL which leads to decreased protein stability and activity.[72,94]

NON-GENETIC FACTORS:

DIET:

- Diet rich in trans-unsaturated fat markedly decreases PON1 activity.[65,69,73]
- Consumption of degraded cooking oil lowers PON1 activity[95]
- Alcohol and vitamin C and E elevate its activity.[69,96]
- Oleic acid from olive oil increases PON-1's activity.
- Nutritional antioxidants such as carotenoids (lycopene and beta carotene) and polyphenols (pomegranate, red wine,tea, soy, liqourice root& grapes) increases PON-1 activity.[65,69,73]

ENVIRONMENTAL FACTORS:

- Oxidative stress leads to enzyme inactivation.[97]
- Smoking decreases PON1 activity.[97]

- Exposure to chemicals like organophosphorus causes long term decrease in PON1 activity.[69,73]
- Statins and fibrates increases PON1 activity by upregulating the gene expression.[98,99]
- Decrease in serum PON1 activity may occur as a part of inflammatory response by cytokines.[100]

AIMS AND OBJECTIVES

AIM:

To estimate the levels of serum Paraoxonase-1 in patients with metabolic syndrome and to compare the levels with healthy controls.

OBJECTIVES:

1) To estimate the relationship of serum paraoxonase-1activity with the components of fasting glucose levels, lipid profile, blood pressure and waist circumference.

2) To correlate serum Paraoxonase level with the malondialdehyde levels.

3) To evaluate the association of Paraoxonase -1 activity & malondialdehyde levels with components of metabolic syndrome.

MATERIALS AND METHODS

The study was conducted, at Thanjavur Medical College, Thanjavur, after getting approval from the ethical committee of Thanjavur medical college, Thanjavur.

Hundred subjects were chosen for the study. Both males and females in the age group of 21-60 years were included and informed consent were obtained from all of them. The study population includes 2 groups:

Control group consists of 50 healthy individuals and Study group consists of 50 patients with metabolic syndrome.

INCLUSION CRITERIA:-

Patients with components of metabolic syndrome were included in the study

EXCLUSION CRITERIA:-

- Acute and chronic infections
- Chronic inflammatory disorders
- Thyroid disorders
- Smoking
- Chronic Alcoholism
- Liver diseases
- Renal failure

SAMPLE COLLECTION:-

Under aseptic precautions, fasting venous blood sample of 6ml was collected from each subject .The vacutainers containing the blood samples were kept at room temperature for 30 min and were centrifuged at 2000 rpm for 15 minutes for clear separation of serum .The following parameters were estimated, immediately after the serum was separated.

1) Paraoxonase activity

2) Glucose

5) Total cholesterol

6) HDL cholesterol

7) Triacyl glycerol

8) Malondialdehyde

LDL and VLDL cholesterol levels were calculated from the estimated parameters.

Materials used for study:

1. Proforma – to record the anthropometric measurements of the subjects and the clinical findings.

2. Portable weighing machine – to record the body weight in kilograms.

3. Non elastic inch tape - to measure Waist circumference in centimeters.

4. Standardized mercury sphygmomanometer – to record the Blood Pressure.

ESTIMATION OF SERUM PON-1 ACTIVITY:

PON-1 activity was estimated using the paraoxon (O,O diethyl-O-4 nitro phenyl phosphate) as the substrate for hydrolysis. The chemicals used were of analytical reagent grade from Sigma chemicals.

PRINCIPLE:

Serum Paraoxonase1 hydrolyses paraoxon in the presence of calcium, at pH 8.0 at 25° C and releases Para nitro phenol (P-NP).The liberated P-NP is measured, and the activity of PON can be calculated using the molar absorption of P-NP in a kinetic assay.The absorbance was measured at 405nm.One unit (1U)of PON activity is defined as 1micromol of para nitro phenol formed per minute per litre at 25˚c and the activity was expressed as U/L of serum.

$$\text{Paraoxon} + H_2O \xrightarrow{\text{Paraoxonase}} \text{Di ethyl phosphate} + \text{P-nitro phenol}$$

REAGENTS:-

The assay mixture consists of 2.2mM paraoxone substrate in 0.1M Tris Hcl buffer, PH 8.0 containing 2Mm $CaCl_2$.

REAGENT PREPARATION:-

BUFFER:-

- 0.1M TRIS: 12.114gm of Tris is dissolved in 1000 ml of distilled water.
- 0.1M Hcl: 1ml of 10 molar solution of Hcl in 99ml of distilled water.
- 2mM $CaCl_2$: 294.04mg of Calcium chloride is dissolved along with Tris.

To prepare this buffer, 90ml of Tris solution is taken and 0.1M Hcl is added till Ph 8.0 is obtained. The buffer solution is refrigerated.

SUBSTRATE PREPARATION:-

2mM paraoxon is needed. To achieve this 3µl of the paraoxon is added to 6.355ml of the buffer. This is freshly prepared.

MOLAR ABSORPTIVITY OF PARA NITRO PHENOL:-

The micro molar absorptivity of P-nitro phenol is measured as follows

- Molecular weight of P-NP=139.11 g /mol
- 1 micromole=139.1µgm/L

The amount is weighed and dissolved in the buffer and the absorption is read at 405nm in the semi auto analyser.

As per Beer's law: $A = \in b\ c$

A=Absorption, $\in$ = Absorptivity, b = path length, c = concentration of the substance.

$\in$ = A/ bc, where b=1cm,c=1 µmol.

Micro molar absorptivity=0.034/ 1x1

$$\text{Enzyme activity U/L} = \frac{\text{Delta absorbance / min x Total volume x1000}}{\text{Sample volume x micromolar absorptivity x path length}}$$

- Factor can be calculated by the formula

$$F = \frac{\text{Total volume x1000}}{\text{Sample volume x micro molar absorptivity x path length}}$$

= 550x1000/ 50x0.034 x1

Factor =374

Thus enzyme activity U/L=Δ Absorbance x374

ASSAY PARAMETERS:-

- MODE :- Kinetic mode
- WAVELENGTH:- 405nm
- SAMPLE VOLUME:- 50µl
- REAGENT VOLUME:-500µl
- LAG TIME:-60 sec
- KINETIC INTERVAL:-180sec
- NUMBER OF READINGS:- 3
- FACTOR:-374
- REACTION TEMPERATURE:-25˚C
- REACTION DIRECTION:-Increasing
- UNITS:- U/ L

ASSAY PROCEDURE:-

500µl of the reagent and 50µl of the sample were taken, mixed well and read at 405nm using the semi auto analyzer.

NORMAL RANGE: 90-200 U/L **INTERFERENCES:** Haemolysed serum

ESTIMATION OF TOTAL CHOLESTEROL

METHOD: Cholesterol oxidase-Peroxidase Enzymatic, endpoint method.

PRINCIPLE:

The free cholesterol, liberated from the cholesterol esters by cholesterol esterase, is oxidised by cholesterol oxidase to cholestenone with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4 amino antipyrine and a phenolic compound in the presence of peroxidase to yield a red coloured complex.

1. Cholesterol ester +water $\xrightarrow{\text{CHE}}$ cholesterol + fatty acid

2. Cholesterol + oxygen $\xrightarrow{\text{CHO}}$ cholest-4-en-3one +H_2O_2

3. 2 H_2O_2 + 4AAP+ phenol $\xrightarrow{\text{POD}}$ quinoneimine dye + $4H_2O$

•CHE- Cholesterol esterase • CHO- Cholesterol oxidase

•4AAP- 4 amino antipyrine • POD- Peroxidase

Absorbance of quinoneimine formed is directly proportional to the concentration of cholesterol.

Reconstituted reagent:

Dissolve the contents of one bottle of the reagent-1(Chromogen/Enzyme) with one bottle of reagent-1A (BUFFER)

ASSAY PROCEDURE: Taken three test tubes and marked as Blank, Standard,Test.Taken working Reagent of about 1000 µl into each test tubes. Then 10 µl of Distilled water was added in the blank,10 µl of std (200mg/dl) was added in the test tube marked as standard and in the tube marked as test - 10 µl of patient sample was added.

Mixed well and incubated for 10 min at room temperature. The absorbance of the test and standard were read against reagent blank at wavelength of 505 nm.

CALCULATION:

$$\text{Cholesterol} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard(mg/dl)}$$

REFERENCE RANGE: 150-200 mg/dl

Linearity –up to 750 mg/dl,Sensitivity-1mg/dl

INTERFERENCE: Hb upto 200mg/dl, Ascorbate upto 12mg/dl, Bilirubin upto 10mg/dl and Triglycerides upto700 mg/dl do not interfere with the test

ESTIMATION OF TRIGLYCERIDES

METHOD: GPO-PAP method, endpoint

METHODOLOGY:

Colorimetric, enzymatic method with glycerol phosphate oxidase.

PRINCIPLE:

TGL reacts with water in presence of Lipoprotein lipase it forms Glycerol and free fatty acids. Glycerol reacts with ATP in the presence of Glycerol Kinase it forms Glycerol 3 phosphate and ADP. Glycerol 3 phosphate reacts with oxygen in presence of Glycerol 3 phosphate oxidase it forms Dihydroxy Acetone Phosphate and Hydrogen Peroxide. Hydrogen Peroxide reacts with 4Amino Anti Pyrine and 3,5Dichloro-2Hydroxy Benzene Sulfonate in presence of peroxidase Quinoneimine dye and two water molecules are formed.

The intensity of Quinoneimine dye formed is proportional to the concentration of Triglycerides present in the sample, when measured at 505 nm (500-540nm).

REAGENT PREPARATION:

The working reagent was prepared by mixing 4 parts of R1(Enzymes/chromogen) with 1 part of R2(Buffer).Stable for 90 days at 2-8 ˚C.

Sample: Unhemolysed serum collected after 12 hrs of fasting.

ASSAY PROCEDURE:

Taken three test tubes and marked as Blank, Standard,Test.Taken working Reagent of about 1000 µl into each test tubes. Then 10 µl of Distilled water was added in the blank,10 µl of std (200mg/dl) was added in the test tube marked as standard and in the tube marked as test - 10 µl of patient sample was added.

Mixed and incubated for 10min,at room temperature . Absorbance were read at 505nm for standard and sample against reagent blank.

CALCULATION:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

REFERENCE VALUES:

Serum/plasma	37°C
Normal fasting level	25-160mg/dl

Linearity – upto 1000mg/dl,Sensitivity- 2mg/dl

INTERFERENCE:-

Hb upto300mg/dl,

Ascorbate upto 3mg/dl,

Bilirubin upto 20mg/dl do not interfere with the test.

ESTIMATION OF HDL CHOLESTEROL:

METHOD: Phosphotungstic acid method,endpoint

PRINCIPLE:

Chylomicrons (CM), LDL and VLDL are precipitated from serum or plasma with phospho tungstate in the presence of divalent cations such as Magnesium.The HDL cholesterol remains unaffected in the supernatant and is estimated using cholesterol reagent.

$$\text{Serum/plasma} \xrightarrow{\text{Phosphotungstic acid, } Mg^{2+}} \underset{\text{(Supernatant)}}{\text{HDL}} + \underset{\text{(Precipitate)}}{\text{(LDL+VLDL+CM)}}$$

REAGENT COMPOSITION:

Reagent1: precipitating reagent

Phosphotungstic acid	2.4mmol/l
Magnesium chloride	40mmol/l

HDL cholesterol standard – 25mg/dl

SAMPLE: Unhemolysed serum used

PRECIPITATION:

Precipitation of LDL, VLDL and Chylomicrons done as follows:

Pipette	Volume
Sample	250µl
Precipitating reagent	500µl

Mixed well and the reaction mixture was allowed to stand for 10 min at room temperature, centrifuged at 4000 rpm for 10min and obtain a clear supernatant. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

ASSAY PROCEDURE:

Pipette into tubes marked	Blank	Standard	Test
Cholesterol working reagent	1000µl	1000µl	1000µl
Distilled water	50µl	-	-
HDL standard	-	50µl	-
Supernatant	-	-	50µl

Mixed well and incubated for 10 min at room temperature. The absorbance of the standard and the test samples were read at 505 nm against reagent blank.

CALCULATION

$$\text{HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{conc.of standard} \times \text{dilution factor}$$

$$= \frac{\text{Absorbance of the test}}{\text{Absorbance of the standard}} \times 25 \times 3$$

$$= \frac{\text{Absorbance of the test}}{\text{Absorbance of the standard}} \times 75$$

Linearity-upto 125mg/dl

NORMAL VALUES :

Males- 40to 65mg/dl

Females-45to80mg/dl

INTERFERENCE:

High triglyceride concentration above 300 mg/dl cause interference with the assay.

Bilirubin and ascorbate at high concentrations interfere with precipitation.

Friedewald's formula for calculation of LDL

VLDL=TGL/5,if TGL is less than 400mg/dl

LDL = Total cholesterol – (HDL + VLDL)

Estimation of Urea by Di acetyl Monoxime method

Estimation of creatinine by Jaffe's method.

ESTIMATION OF GLUCOSE

METHOD: Glucose oxidase- peroxidase method, end point/fixed time

PRINCIPLE: Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyses oxidative coupling of 4 –amino antipyrine with phenol to yield a coloured complex of quinoneimine , with absorbance is proportional to the concentration of glucose in the sample.

Glucose standard : 100mg/dl

Specimen: Fresh unhemolysed serum used

ASSAY PROCEDURE:

Taken three test tubes and marked as Blank, Standard,Test.Taken working Reagent of about 1000 µl into each test tubes. Then 10 µl of Distilled water was added in the blank,10 µl of std (100mg/dl) was added in the test tube marked as standard and in the tube marked as test - 10 µl of patient sample was added.

Mixed well and incubated at 37˚C for 5 min. The absorbance of the standard and the test were read against reagent blank at 505nm.

CALCULATION:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard(100mg/dl)}$$

Linearity upto 500mg/dl by endpoint method.

NORMAL VALUES:

- Glucose fasting= 70-110 mg/dl
- Glucose postprandial= 90-140 mg/dl.

Estimation of Malondialdehyde (MDA) by Thio Barbituric Acid reactivity assay method:

Serum MDA was measured by thiobarbituric acid reactivity assay method of Satoh. Serum MDA can be measured by various methods including its reactivity with thiobarbituric acid. In spite of many cross-reacting substances are found to increase the color when MDA is measured by this method, thiobarbituric acid reacting substances (TBARS) is the most widely used method for the estimation of MDA because it is relatively inexpensive and very easy to perform.

Principle:

The proteins are precipitated by trichloroacetic acid. Sulphuric acid hydrolyses the lipid peroxides from the protein to yield a malondialdehyde (MDA). This MDA reacts with thiobarbituric acid (TBA) to produce MDA-TBA adduct. On boiling in water bath, this gives a pink color, which is measured at absorbance of 535 nm using n-butanol as blank.

Reagents:

- Trichloroacetic acid (TCA): 20% solution
- Thiobarbituric acid (TBA): 0.22% solution in 2M sodium sulphate
- Sulphuric acid (0.05 M)

Procedure:

1. In a centrifuge tube, 250µL serum diluted with 250µL distilled water was taken and mixed with 2.5ml 20% TCA and kept for 10 min for precipitation of proteins
2. Precipitated proteins were separated by centrifuge at 3500 rpm for 10 min.
3. The supernatant was discarded and the protein pellet was washed with 2ml of 0.05M sulphuricacid.
4. Precipitate was then incubated with 2ml of 0.05M sulphuricacid and 3ml of TBA reagent in a boiling water bath for 30 min.

5. After 30 min, the centrifuge tubes were cooled immediately under tap water to arrest the reaction.
6. Once the tubes were cooled, 4ml of butanol was added and mixed vigorously using a vortex machine.
7. Further the tubes were centrifuged at 3500 rpm for 10 min to obtain a clear supernatant.
8. Absorbance of supernatant was measured at 530nm using photoelectric calorimeter.

Calculation:

Concentration of MDA in serum = 102.56 × A μmol/L, where A is absorbance value.

Molar extinction coefficient of MDA = 1.56×10^5M/L/cm

Values were expressed inμmoles/L.

Reference range:

Serum MDA: 2-5 μmoles/

MASTER CHART –I CONTROL GROUP																			
S.NO	**AGE yrs**	**SEX**	**SBP**	**DBP**	**W C cm**	**WEIGHT Kg**	**HEIGHT mts**	**BMI Kg/m^2**	**MDA µmol/L**	**PON ACT U/L**	**FBS mg/dl**	**PPBS mg/dl**	**LIPID PROFILE (mg/dl)**					**UREA mg/dl**	**CREA mg/dl**
1	39	F	128	78	84	51	1.58	20.42942	1.025	165	84	120	144	110	45	77	22	26	0.8
2	25	F	120	80	81	48	1.58	19.22769	1.025	211	98	130	131	102	51	59.6	20.4	28	0.9
3	28	F	110	80	81	48	1.55	19.97919	1.025	179	84	124	140	106	51	67.8	21.2	32	1
4	37	F	120	78	80	56	1.54	23.61275	2.05	162	87	128	120	90	52	50	18	28	0.9
5	34	F	124	82	78	67	1.58	26.83865	3.076	143	84	116	165	123	51	89.4	24.6	36	1.2
6	45	F	128	84	83	49	1.59	19.38214	2.05	168	96	130	134	102	48	65.6	20.4	38	1.2
7	35	F	124	82	78	69	1.54	29.09428	6.153	105	85	126	182	139	54	100.2	27.8	28	1
8	58	F	130	78	84	51	1.62	19.43301	2.05	173	99	130	138	101	51	66.8	20.2	28	0.9
9	44	F	118	84	83	69	1.52	29.86496	4.102	118	95	132	162	132	42	93.6	26.4	22	0.6
10	21	F	120	80	80	44	1.59	17.40437	2.05	184	96	136	131	100	50	61	20	28	0.8
11	60	F	128	84	86	62	1.61	23.91883	3.076	149	88	132	174	99	42	112.2	19.8	28	0.7
12	50	F	120	82	84	56	1.54	23.61275	3.076	130	98	138	151	119	45	82.2	23.8	28	0.9
13	52	F	126	84	85	62	1.54	26.14269	4.102	115	99	139	181	131	43	111.8	26.2	34	1
14	44	F	128	80	99	78	1.66	28.306	5.128	119	94	136	184	142	40	115.6	28.4	30	1
15	41	F	126	78	80	46	1.58	18.42653	2.05	174	88	126	135	89	53	64.2	17.8	32	0.8

16	30	F	120	76	78	48	1.62	18.28989	1.025	197	99	135	132	101	45	66.8	20.2	30	0.8
17	46	F	130	84	83	49	1.55	20.39542	1.025	200	97	137	125	102	50	54.6	20.4	28	1
18	54	F	128	80	96	52	1.48	23.73996	3.076	145	98	134	164	133	41	96.4	26.6	34	0.9
19	51	F	128	82	85	52	1.65	19.10009	3.076	128	99	137	141	105	45	75	21	34	0.8
20	53	F	124	80	88	53	1.52	22.93975	4.102	162	98	139	163	121	43	95.8	24.2	32	1
21	42	F	128	80	99	69	1.59	27.29322	5.128	117	92	126	172	142	41	102.6	28.4	28	0.8
22	38	F	126	78	80	46	1.59	18.19548	2.05	171	90	138	152	117	49	79.6	23.4	34	0.8
23	32	F	124	76	78	47	1.61	18.13202	2.05	185	84	112	141	112	51	67.6	22.4	22	0.9
24	43	F	128	80	82	69	1.55	28.72008	5.128	108	98	139	162	130	45	91	26	38	1.2
25	30	F	120	76	78	57	1.56	23.42209	3.076	152	96	138	139	115	50	66	23	30	0.9
26	45	M	126	78	88	65	1.68	23.03005	2.05	155	87	110	131	102	42	68.6	20.4	28	0.8
27	57	M	128	80	99	84	1.74	27.74475	5.128	101	98	126	181	135	41	113	27	30	0.9
28	52	M	136	80	96	69	1.58	27.6398	4.102	112	88	108	178	142	41	108.6	28.4	26	0.9
29	50	M	128	80	95	73	1.68	25.86451	4.102	130	99	132	183	148	45	108.4	29.6	34	1.1
30	35	M	124	82	88	73	1.62	27.81588	4.102	119	84	124	178	148	52	96.4	29.6	30	0.9
31	46	M	130	78	101	63	1.65	23.1405	1.025	195	88	110	134	94	50	65.2	18.8	28	0.9
32	43	M	128	84	89	80	1.78	25.24934	3.076	138	95	130	145	105	43	81	21	30	0.9
33	52	M	130	82	93	74	1.74	24.4418	1.025	193	90	129	138	102	44	73.6	20.4	34	0.8

34	48	M	116	80	99	66	1.73	22.05219	4.102	131	93	131	164	133	46	91.4	26.6	26	0.7
35	25	M	120	80	98	52	1.67	18.64534	1.025	199	84	125	128	99	48	60.2	19.8	22	0.6
36	35	M	124	82	98	63	1.7	21.79931	2.05	163	84	129	127	102	52	54.6	20.4	28	0.8
37	45	M	118	84	92	70	1.69	24.50895	3.076	143	96	138	156	126	44	86.8	25.2	24	0.9
38	38	M	120	78	88	65	1.73	21.71807	2.05	194	86	129	128	96	56	52.8	19.2	26	0.8
39	28	M	110	76	98	62	1.59	24.52435	2.05	160	89	123	131	100	54	57	20	30	1
40	55	M	130	78	101	83	1.72	28.05571	4.102	108	84	121	190	146	40	120.8	29.2	28	0.9
41	42	M	130	84	89	66	1.72	22.30936	2.05	173	94	131	130	94	50	61.2	18.8	34	0.9
42	21	M	120	80	88	62	1.68	21.96712	2.05	175	86	119	142	112	51	68.6	22.4	24	0.6
43	55	M	130	78	92	73	1.62	27.81588	3.076	159	97	138	149	129	46	77.2	25.8	28	0.9
44	23	M	120	80	99	62	1.62	23.62445	2.05	192	78	119	153	113	52	78.4	22.6	24	0.6
45	38	M	126	82	100	72	1.61	27.77671	2.05	163	85	122	156	127	50	80.6	25.4	36	0.8
46	47	M	130	80	89	66	1.74	21.79945	1.025	195	92	132	131	90	54	59	18	38	1
47	34	M	120	76	98	63	1.69	22.05805	2.05	169	86	117	142	113	52	67.4	22.6	36	1.2
48	56	M	130	78	92	74	1.64	27.51338	3.076	150	99	136	152	92	50	83.6	18.4	26	0.6
49	60	M	136	80	98	79	1.63	29.7339	6.153	100	99	135	184	146	42	112.8	29.2	28	1
50	51	M	130	82	94	75	1.7	25.95156	2.05	165	97	133	133	99	48	65.2	19.8	30	0.8

MASTER CHART – II STUDY GROUP

S.NO	AGE	SEX	SBP	DBP	W C	Wt	Ht	BMI	MDA	PON ACT	FBS	PPBS	LIPID PROFILE(mg/dl)					UREA	CREAT
			mm Hg		cms	Kg	mts		µmoles/L	U/L	mg/dl	mg/dl	T.CHO	TGL	HDL	LDL	VLDL	mg/dl	mg/dl
1	36	F	128	82	106	95	1.72	32.11	7.179	67.3	108	129	195	156	36	127.8	31.2	20	0.6
2	49	F	138	86	119	80	1.54	33.73	9.23	45.6	110	143	211	180	41	134	36	28	0.9
3	33	F	128	80	108	81	1.58	32.45	7.179	66.7	92	104	192	152	40	121.6	30.4	30	1
4	48	F	140	90	117	85	1.65	31.22	8.204	52.6	111	151	207	166	36	137.8	33.2	26	1
5	23	F	130	82	112	82	1.58	32.85	7.179	62.9	92	110	195	154	39	125.2	30.8	26	0.8
6	28	F	120	80	103	79	1.59	31.25	6.156	76.9	100	163	185	144	38	118.2	28.8	28	0.9
7	38	F	136	86	114	86	1.6	33.59	8.204	56.4	105	145	209	168	35	140.4	33.6	32	0.8
8	48	F	138	88	117	82	1.56	33.69	9.23	41.2	102	147	202	184	32	133.2	36.8	34	1
9	54	F	138	88	115	96	1.69	33.61	8.204	51.4	109	157	207	162	40	134.6	32.4	32	0.8
10	41	F	130	86	103	79	1.59	31.25	6.156	77.5	104	146	182	142	45	108.6	28.4	28	0.9
11	60	F	140	90	132	102	1.65	37.47	12.256	19.2	125	193	256	205	28	187	41	32	0.8
12	21	F	120	80	97	79	1.59	31.25	5.128	75.4	100	131	175	132	44	104.6	26.4	24	0.6
13	49	F	138	86	119	89	1.64	33.09	8.204	55.7	120	163	208	168	39	135.4	33.6	22	0.8

14	25	F	128	80	96	80	1.62	30.48	5.128	85.7	100	136	172	132	45	100.6	26.4	36	1.1
15	42	F	130	86	102	78	1.55	32.47	6.156	78.4	103	135	184	140	41	115	28	30	0.9
16	57	F	138	88	124	96	1.68	34.01	11.281	30.4	114	168	224	198	28	156.4	39.6	34	1
17	56	F	138	86	122	88	1.59	34.81	10.256	34.6	113	162	216	192	30	147.6	38.4	24	0.7
18	45	F	140	88	118	86	1.6	33.59	9.23	49.4	110	157	213	185	38	138	37	28	1
19	50	F	140	90	128	92	1.58	36.85	11.281	29.4	124	187	236	200	30	166	40	34	1
20	31	F	120	80	85	83	1.68	29.41	4.104	80.2	100	140	155	126	44	85.8	25.2	34	1
21	45	F	130	86	90	80	1.59	31.64	6.156	76.4	101	142	178	129	37	115.2	25.8	29	0.9
22	28	F	126	80	109	83	1.6	32.42	7.179	63.2	105	137	192	153	38	123.4	30.6	30	0.8
23	32	F	120	80	87	70	1.55	29.14	5.128	85.4	85	178	170	151	43	96.8	30.2	26	0.8
24	53	F	138	88	115	97	1.7	33.56	8.204	53.8	121	186	205	172	40	130.6	34.4	34	0.9
25	44	F	120	80	93	76	1.55	31.63	6.156	78.7	101	133	179	138	45	106.4	27.6	36	1
26	46	M	135	80	112	97	1.69	33.96	9.23	48.2	115	150	209	172	33	141.6	34.4	28	0.9
27	55	M	140	90	110	82	1.58	32.85	8.204	52.7	111	152	203	161	32	138.8	32.2	36	1.2
28	52	M	140	82	113	85	1.62	32.39	7.179	68.4	120	161	193	156	34	127.8	31.2	32	0.8
29	38	M	130	84	116	88	1.61	33.95	9.23	47.5	108	142	209	172	32	142.6	34.4	40	1.1
30	45	M	132	86	100	86	1.68	30.47	5.128	80.3	102	132	172	138	34	110.4	27.6	32	0.9
31	60	M	140	90	131	98	1.64	36.44	12.256	21.2	124	183	252	201	28	183.8	40.2	36	1.1
32	28	M	120	80	98	78	1.59	30.85	5.128	86.9	100	122	175	132	37	111.6	26.4	28	0.9

33	56	M	144	86	117	92	1.65	33.79	9.23	45.8	108	128	210	175	32	143	35	32	0.9
34	34	M	130	86	106	89	1.69	31.16	6.156	74.2	107	130	185	146	36	119.8	29.2	32	0.9
35	42	M	138	88	118	102	1.72	34.48	10.256	33.2	112	172	214	186	30	146.8	37.2	24	0.9
36	57	M	142	90	108	89	1.65	32.69	7.179	68.9	109	126	196	152	37	128.6	30.4	34	0.9
37	35	M	130	80	116	90	1.7	31.14	6.156	72.4	106	127	177	138	34	115.4	27.6	28	1
38	48	M	138	86	105	90	1.73	30.07	6.156	73.9	104	135	186	147	35	121.6	29.4	28	0.9
39	35	M	130	78	105	84	1.62	32.01	7.179	66.2	102	132	194	153	35	128.4	30.6	34	0.9
40	52	M	142	88	112	98	1.71	33.51	9.23	48.5	109	142	210	176	34	140.8	35.2	28	1
41	43	M	144	90	119	105	1.75	34.29	10.256	39.6	120	184	215	190	30	147	38	28	0.8
42	47	M	136	86	113	98	1.7	33.91	8.204	57.7	102	126	205	164	33	139.2	32.8	32	0.8
43	51	M	144	92	123	102	1.72	34.48	11.289	32.9	103	131	223	195	29	155	39	28	0.8
44	21	M	120	80	96	80	1.69	28.01	4.102	94.2	100	132	165	129	43	96.2	25.8	34	1
45	45	M	138	86	119	96	1.69	33.61	9.23	44.2	116	156	209	178	32	141.4	35.6	28	0.7
46	25	M	120	78	105	89	1.67	31.91	6.156	77.4	92	102	187	144	36	122.2	28.8	28	0.7
47	55	M	138	88	120	99	1.7	34.26	10.256	33.2	119	158	219	189	27	154.2	37.8	26	1.1
48	38	M	128	78	107	95	1.71	32.49	7.179	67.2	108	132	194	155	35	128	31	28	0.9
49	50	M	140	90	114	95	1.69	33.26	8.204	50.6	113	158	204	166	32	138.8	33.2	24	0.7
50	23	M	128	80	99	80	1.62	30.48	5.128	86.7	103	139	178	133	38	113.4	26.6	28	1

TABLE 1 :Descriptive Statistics of Study and Control Groups

Variables	Control (n=50)				Study (n=50)			
	Min.	**Max.**	**Mean**	**S.D.**	**Min.**	**Max.**	**Mean**	**S.D.**
AGE	21	60	42.26	10.6939	21.0	60.0	42.340	11.1604
SBP	110	140	125.84	6.65	120	144	133.18	7.575
DBP	76	85	80.14	2.408	78	92	84.76	4.118
WC	78	101	89.1	7.627	85	132	110.26	10.779
WEIGHT	44	84	62.64	10.883	70	105	88.22	8.237
HT(mt)	1.48	1.78	1.6258	0.07106	1.54	1.75	1.6418	.05749
BMI	17.40	29.86	23.65	3.58	28.01	37.47	32.68	1.88
MDA	1.025	6.153	2.809	1.403	4.102	12.256	7.813	2.097
PON	100.00	211.00	154.84	30.71	19.20	94.20	59.33	19.07
FBS	78	99	91.68	6.001	85	125	107.36	8.806
PPBS	108	139	128.5	8.36	102	193	145.90	21.100
T.CHO	120	190	150.54	19.775	155	256	198.64	20.632
TGL	89	148	115.12	18.022	126	205	161.54	22.117
HDL	40	56	48.36	4.737	28	46	35.38	4.607
LDL	50	120.8	79.156	19.59239	91.8	187.0	130.952	20.4404
VLDL	17.8	29.6	23.024	3.6045	25.2	41.0	32.308	4.4234

TABLE 2

AGE & SEX DISTRIBUTION BETWEEN STUDY & CONTROL GROUPS

	Control (n=50)	Study (n=50)	Total (n=100)
Age			
21-40 yrs	20(40%)	19(36%)	39(39%)
41 to 60 yrs	30(60%)	31(62%)	61(61%)

Sex			
Male (Control)	25 (50%)	0	25 (25%)
Female (Control)	25 (50%)	0	25 (25%)
Male (Study)	0	25 (50%)	25 (25%)
Female (Study)	0	25 (50%)	25 (25%)

TABLE 3

STATISTICAL ANALYSIS OF S.PARAOXONASE-1 BETWEEN STUDY & CONTROL GROUPS

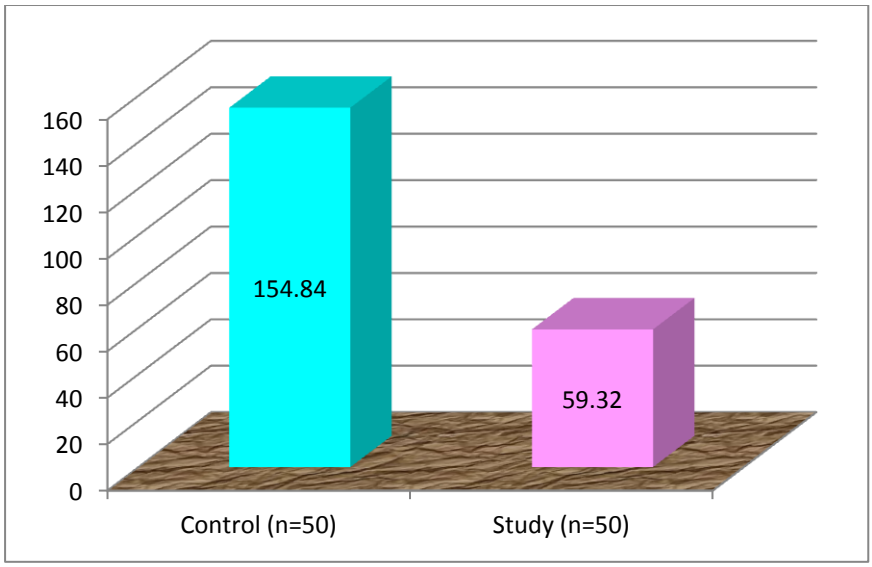
T-TEST			
PARAOXONASE-1	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control (n=50)	154.84	30.71	.000<0.05 **Significant**
Study (n=50)	59.32	19.0	

TABLE 4

STATISTICAL ANALYSIS OF MALONDIALDEHYDE LVELS BETWEEN CONTROL AND STUDY GROUP

T-TEST			
MALONDIALDEHYDE	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control (n=50)	2.809	1.40	.000<0.05 **Significant**
Study (n=50)	7.81	2.09	

BAR CHART 1 : COMPARISON OF SERUM PARAOXONASE-1 ACTIVITY BETWEEN CONTROL AND STUDY GROUP



BAR CHART 2 : COMPARISON OF MALONDIALDEHYDE BETWEEN CONTROL AND STUDY GROUP

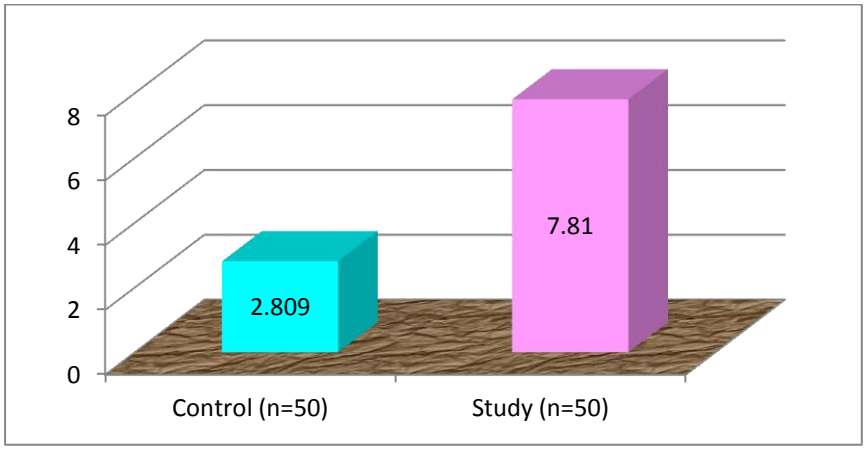


TABLE 5

STATISTICAL ANALYSIS OF BMI BETWEEN CONTROL AND STUDY GROUP

T-TEST			
BMI	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control (n=50)	23.653	3.58	.000<0.05 **Significant**
Study (n=50)	32.68	1.88	

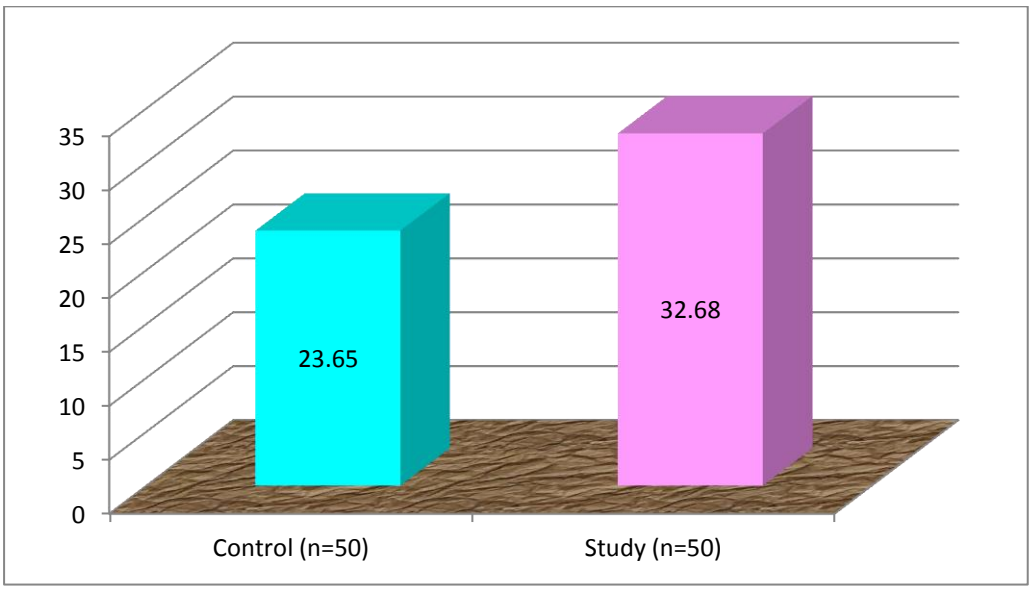
TABLE 6

STATISTICAL ANALYSIS OF WAIST CIRCUMFERANCE BETWEEN CONTROL AND STUDY GROUP

T-TEST			
WAIST CIRCUMFERENCE	**MEAN**	**S.D**	**STAISTICAL INFERENCE**
Control (n=50)	89.1	7.62	.000<0.05 **Significant**
Study (n=50)	110.26	10.77	

BAR CHART 3 : COMPARISON OF BMI

BETWEEN CONTROL AND STUDY GROUP



BAR CHART 4 : COMPARISON OF WAIST CIRCUMFERANCE

BETWEEN CONTROL AND STUDY GROUP

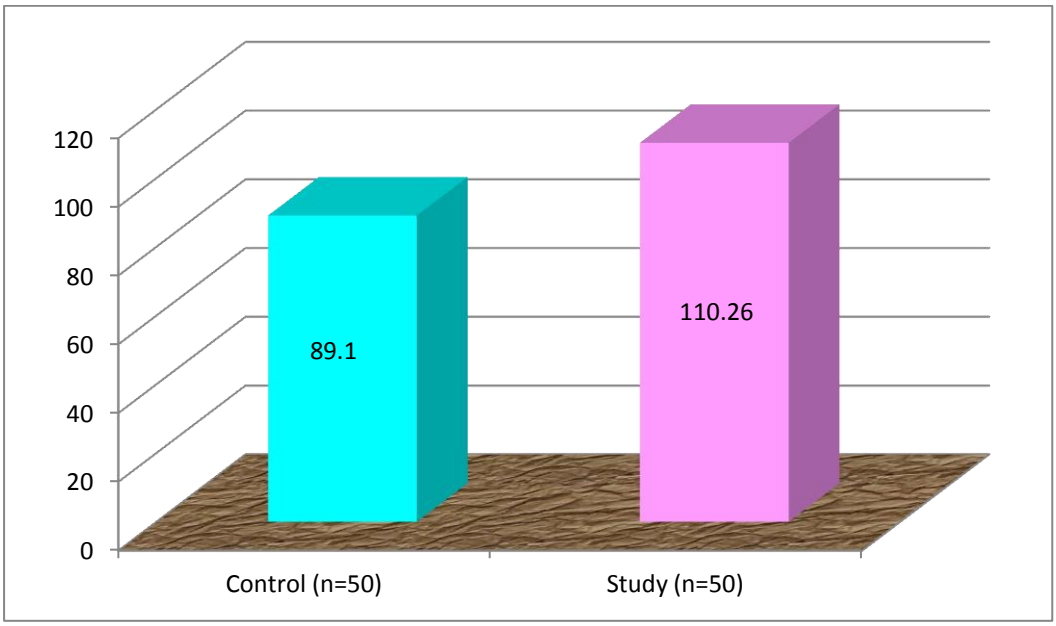


TABLE 7

STATISTICAL ANALYSIS OF FBS & PPBS

BETWEEN CONTROL AND STUDY GROUP

T- TEST			
FBS	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control (n=50)	91.68	6.00	.000<0.05 **Significant**
Study (n=50)	107.36	8.80	
PPBS			
Control (n=50)	128.5	8.36	.000<0.05 **Significant**
Study (n=50)	145.90	21.10	

BARCHART 5: COMPARISON OF FBS & PPBS

BETWEEN CONTROL AND STUDY GROUP

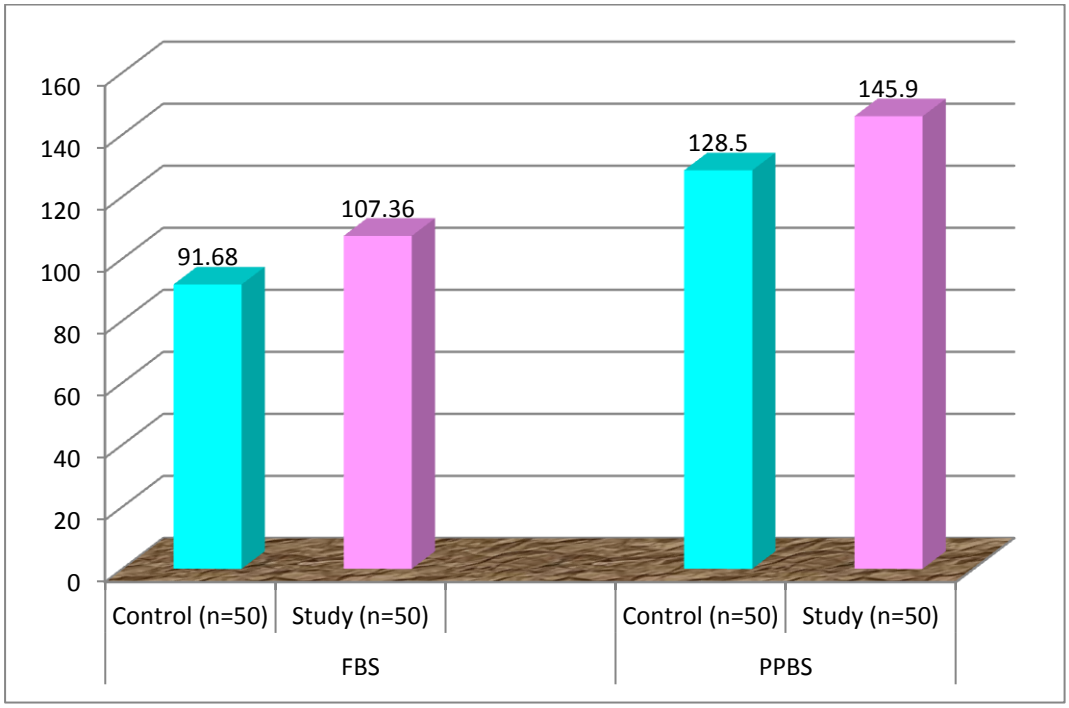


TABLE 8

STATISTICAL ANALYSIS OF SYSTOLIC & DIASTOLIC BP BETWEEN CONTROL AND STUDY GROUP

T- TEST			
SYSTOLIC BP	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control (n=50)	125.84	6.65	.000<0.05 **Significant**
Study (n=50)	133.18	7.575	
DIASTOLIC BP			
Control (n=50)	80.14	2.408	.000<0.05 **Significant**
Study (n=50)	84.76	4.118	

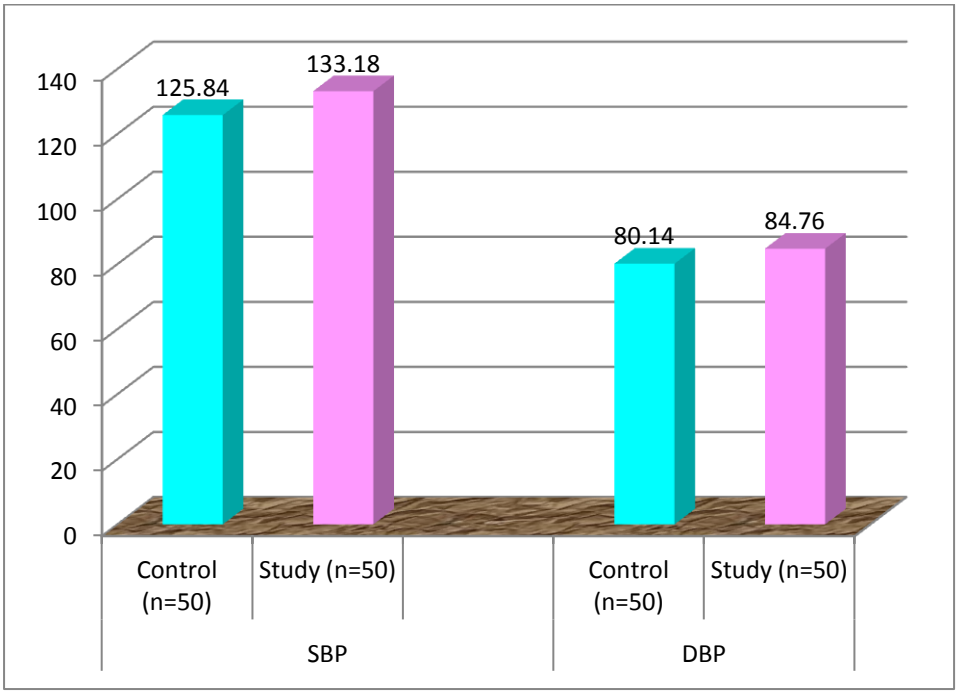
TABLE 9

STATISTICAL ANALYSIS OF TGL BETWEEN CONTROL AND STUDY GROUP

T – TEST			
TGL	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control (n=50)	115.12	18.02	.000<0.05 **Significant**
Study (n=50)	161.54	22.11	

BARCHART 7: COMPARISON OF SBP& DBP(mmHg)

BETWEEN CONTROL AND STUDY GROUP



BAR CHART 8 : COMPARISON OF TGL

BETWEEN CONTROL AND STUDY GROUP

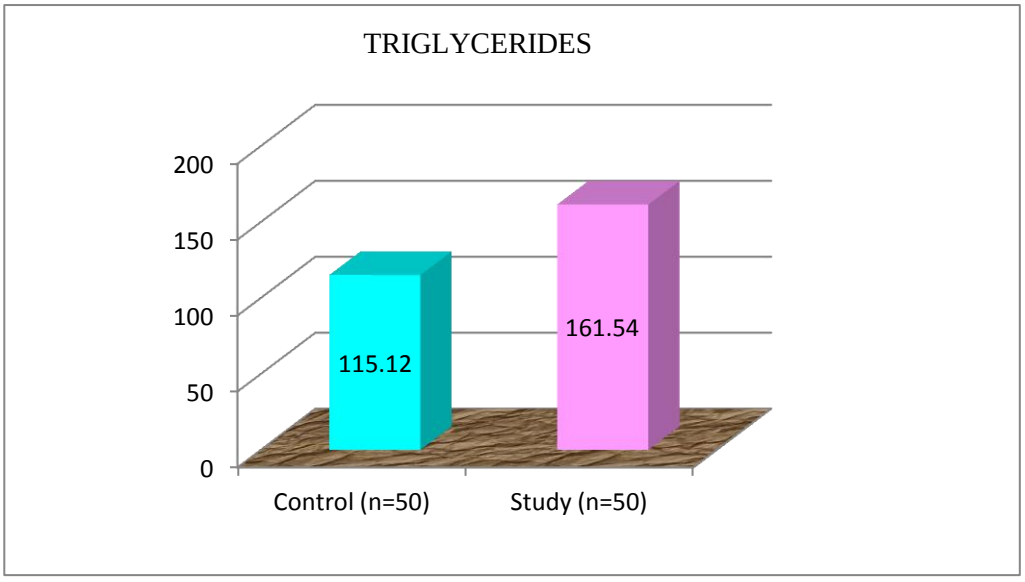


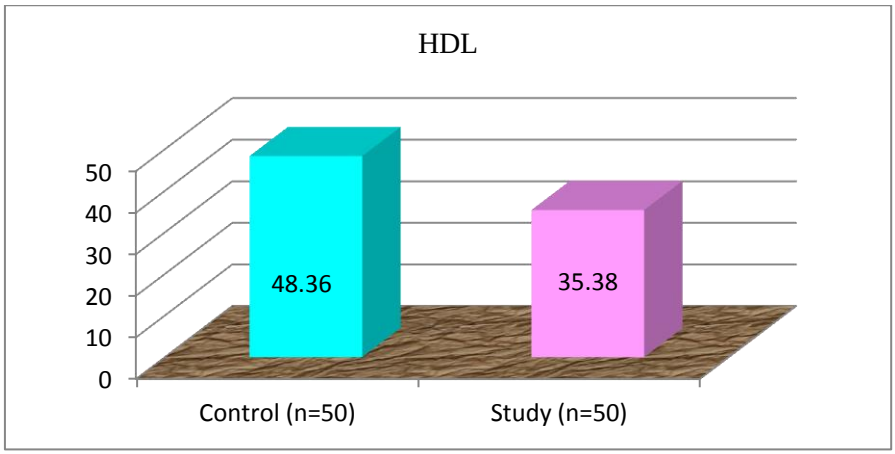
TABLE 10

STATISTICAL ANALYSIS OF T.CHOL, HDL, VLDL, LDL BETWEEN CONTROL AND STUDY GROUP

T– TEST			
T.CHOL	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control (n=50)	150.54	19.77	.000<0.05 **Significant**
Study (n=50)	198.64	20.63	
HDL			
Control (n=50)	48.36	4.73	.000<0.05 **Significant**
Study (n=50)	35.38	4.60	
VLDL			
Control (n=50)	23.024	3.6	.000<0.05 **Significant**
Study (n=50)	32.30	4.42	
LDL			
Control (n=50)	79.156	19.59	.000<0.05 **Significant**
Study (n=50)	130.95	20.44	

BAR CHART 9 : COMPARISON OF HDL

BETWEEN CONTROL AND STUDY GROUP



BAR CHART 10 :COMPARISON OF T.CHOL, VLDL, LDL

BETWEEN CONTROL AND STUDY GROUP

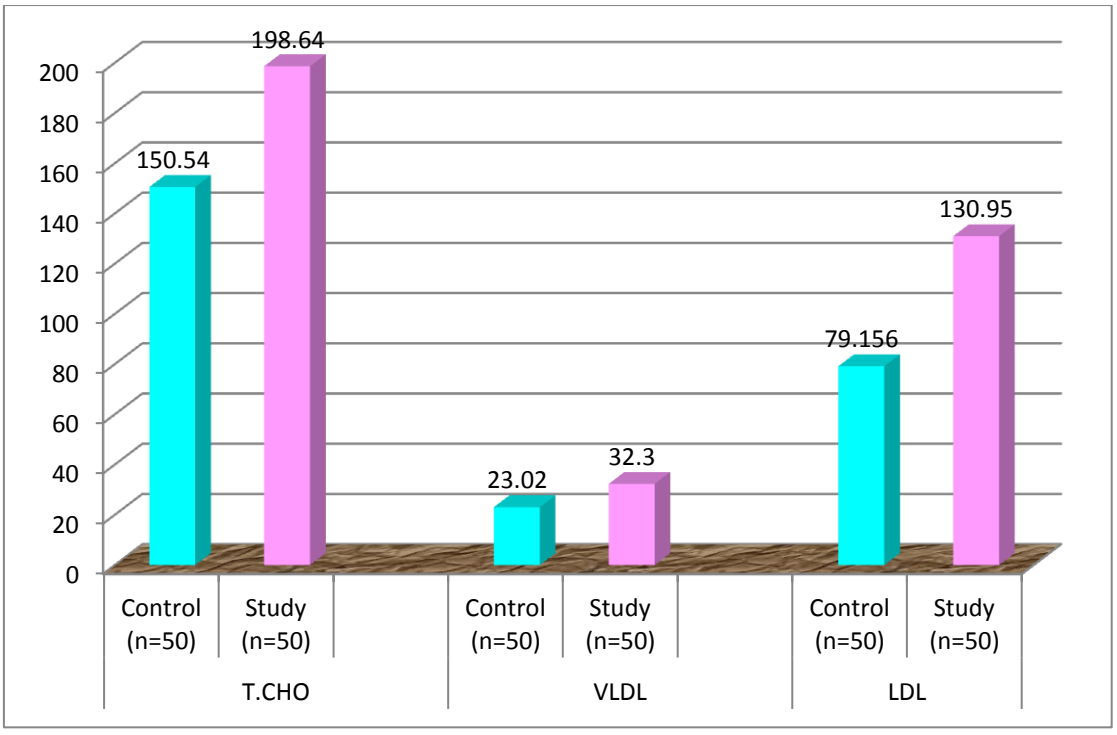


TABLE 11

STATISTICAL ANALYSIS OF PARAOXONASE BETWEEN 3, 4 & 5 COMPONENTS OF METABOLIC SYNDROME.

PARAOXONASE-I ACTIVITY	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11527.77	3	3842.593	28.108	.000(statistically significant)

TABLE 12

PEARSONS CORRELATION BETWEEN PARAOXONASE AND OTHER STUDY PARAMETERS

PON	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	-.260**	P < 0.01 Significant
SBP	-.650**	P <0.01 Significant
DBP	-.663**	P < 0.01 Significant
WC	-.907**	P < 0.01 Significant
WEIGHT	-.713**	P < 0.01 Significant
BMI	-.944**	P < 0.01 Significant
MDA	-.955**	P < 0.01 Significant
FBS	-.773**	P < 0.01 Significant
PPBS	-.575**	P < 0.01 Significant
T.CHOL	-.931**	P < 0.01 Significant
TGL	-.918**	P < 0.01 Significant
HDL	.872**	P < 0.01 Significant
LDL	-.937**	P < 0.01 Significant
VLDL	-.962**	P < 0.01 Significant

TABLE 13

PEARSONS CORRELATION BETWEEN MDA AND OTHER STUDY PARAMETERS

MDA	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	.345**	P < 0.01 Significant
SBP	.728**	P < 0.01 Significant
DBP	.722**	P < 0.01 Significant
WC	.915**	P < 0.01 Significant
WEIGHT	.711**	P < 0.01 Significant
HEIGHT	.180	P > 0.05 not Significant
BMI	.913**	P < 0.01 Significant
PON	-.955**	P < 0.01 Significant
PPBS	.562**	P < 0.01 Significant
FBS	.816**	P < 0.01 Significant
CHOL	.963**	P < 0.01 Significant
TGL	.962**	P < 0.01 Significant
HDL	-.875**	P < 0.01 Significant
LDL	.960**	P < 0.01 Significant
VLDL	.962**	P < 0.01 Significant

TABLE 14

PEARSONS CORRELATION BETWEEN BMI AND OTHER STUDY PARAMETERS

BMI	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	.255*	P < 0.05 Significant
SBP	.638**	P < 0.01 Significant
DBP	.635**	P < 0.01 Significant
WC	.870**	P < 0.01 Significant
WEIGHT	.664**	P < 0.01 Significant
HEIGHT	-.064	P > 0.05 not Significant
MDA	.913**	P < 0.01 Significant
PON	-.944**	P < 0.01 Significant
FBS	.723**	P < 0.01 Significant
PPBS	.495**	P < 0.01 Significant
CHOL	.905**	P < 0.01 Significant
TGL	.881**	P < 0.01 Significant
HDL	-.818**	P < 0.01 Significant
LDL	.905**	P < 0.01 Significant
VLDL	.881**	P < 0.01 Significant

TABLE 15

PEARSONS CORRELATION BETWEEN

TGL AND OTHER STUDY PARAMETERS

TGL	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	.331**	P < 0.01 Significant
SBP	.702**	P < 0.01 Significant
DBP	.696**	P < 0.01 Significant
WC	.901**	P < 0.01 Significant
WEIGHT	.680**	P < 0.01 Significant
HEIGHT	-.179	P > 0.05 not Significant
BMI	.881**	P < 0.01 Significant
MDA	.962**	P < 0.01 Significant
PON	-.918**	P < 0.01 Significant
FBS	.759**	P < 0.01 Significant
PPBS	.613**	P < 0.01 Significant
CHOL	.964**	P < 0.01 Significant
HDL	-.857**	P < 0.01 Significant
LDL	.949**	P < 0.01 Significant
VLDL	1.000**	P < 0.01 Significant

TABLE 16

PEARSONS CORRELATION BETWEEN

HDL AND OTHER STUDY PARAMETERS

HDL	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	-.368**	P < 0.01 Significant
SBP	-.702**	P < 0.01 Significant
DBP	-.660**	P < 0.01 Significant
WC	-.741**	P < 0.01 Significant
WEIGHT	-.535**	P < 0.01 Significant
HEIGHT	-.013	P > 0.05 not Significant
BMI	-.818**	P < 0.01 Significant
MDA	-.875**	P < 0.01 Significant
PON	.872**	P < 0.01 Significant
FBS	-.783**	P < 0.01 Significant
PPBS	-.582**	P < 0.01 Significant
CHOL	-.864**	P < 0.01 Significant
TGL	-.857**	P < 0.01 Significant
LDL	-.912**	P < 0.01 Significant
VLDL	-.857**	P < 0.01 Significant

TABLE 17

PEARSONS CORRELATION BETWEEN WAIST CIRCUMFERENCE AND OTHER STUDY PARAMETERS

WC	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	.650**	P < 0.01 Significant
SBP	.780**	P < 0.01 Significant
DBP	.645**	P < 0.01 Significant
WEIGHT	.695**	P < 0.01 Significant
HEIGHT	.222	P > 0.05 not Significant
BMI	.870**	P < 0.01 Significant
MDA	.915**	P < 0.01 Significant
PON	-.907**	P < 0.01 Significant
FBS	.734**	P < 0.01 Significant
PPBS	.466**	P < 0.01 Significant
CHOL	.926**	P < 0.01 Significant
TGL	.901**	P < 0.01 Significant
HDL	-.741**	P < 0.01 Significant
LDL	.906**	P < 0.01 Significant
VLDL	.901**	P < 0.01 Significant

TABLE 18

PEARSONS CORRELATION BETWEEN

FBS AND OTHER STUDY PARAMETERS

FBS	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	.422**	P < 0.01 Significant
SBP	.707**	P < 0.01 Significant
DBP	.680**	P < 0.01 Significant
WC	.734**	P < 0.01 Significant
WEIGHT	.658**	P < 0.01 Significant
HEIGHT	.322*	P > 0.05 Significant
BMI	.723**	P < 0.01 Significant
MDA	.816**	P < 0.01 Significant
PON	-.773**	P < 0.01 Significant
PPBS	.701**	P < 0.01 Significant
CHOL	.764**	P < 0.01 Significant
TGL	.759**	P < 0.01 Significant
HDL	-.783**	P < 0.01 Significant
LDL	.783**	P < 0.01 Significant
VLDL	.759**	P < 0.01 Significant

TABLE 19

PEARSONS CORRELATION BETWEEN

SBP AND OTHER STUDY PARAMETERS

SBP	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	.643**	P < 0.01 Significant
DBP	.837**	P < 0.01 Significant
WC	.780**	P < 0.01 Significant
WEIGHT	.654**	P < 0.01 Significant
HEIGHT	.069	P > 0.05 not Significant
BMI	.638**	P < 0.01 Significant
MDA	.728**	P < 0.01 Significant
PON	-.650**	P < 0.01 Significant
FBS	.707**	P < 0.01 Significant
PPBS	.436**	P < 0.01 Significant
CHOL	.711**	P < 0.01 Significant
TGL	.702**	P < 0.01 Significant
HDL	-.702**	P < 0.01 Significant
LDL	.723**	P < 0.01 Significant
VLDL	.702**	P < 0.01 Significant

TABLE 20

PEARSONS CORRELATION BETWEEN

DBP AND OTHER STUDY PARAMETERS

DBP	CORRELATION VALUE	STATISTICAL INFERENCE
AGE	.471**	P < 0.01 Significant
SBP	.745**	P < 0.01 Significant
WC	.645**	P < 0.01 Significant
WEIGHT	.530**	P < 0.01 Significant
HEIGHT	.206	P > 0.05 not Significant
BMI	.635**	P < 0.01 Significant
MDA	.722**	P < 0.01 Significant
PON	-.663**	P < 0.01 Significant
FBS	.680**	P < 0.01 Significant
PPBS	.511**	P < 0.01 Significant
CHOL	.696**	P < 0.01 Significant
TGL	.696**	P < 0.01 Significant
HDL	-.660**	P < 0.01 Significant
LDL	.700**	P < 0.01 Significant
VLDL	.696**	P < 0.01 Significant

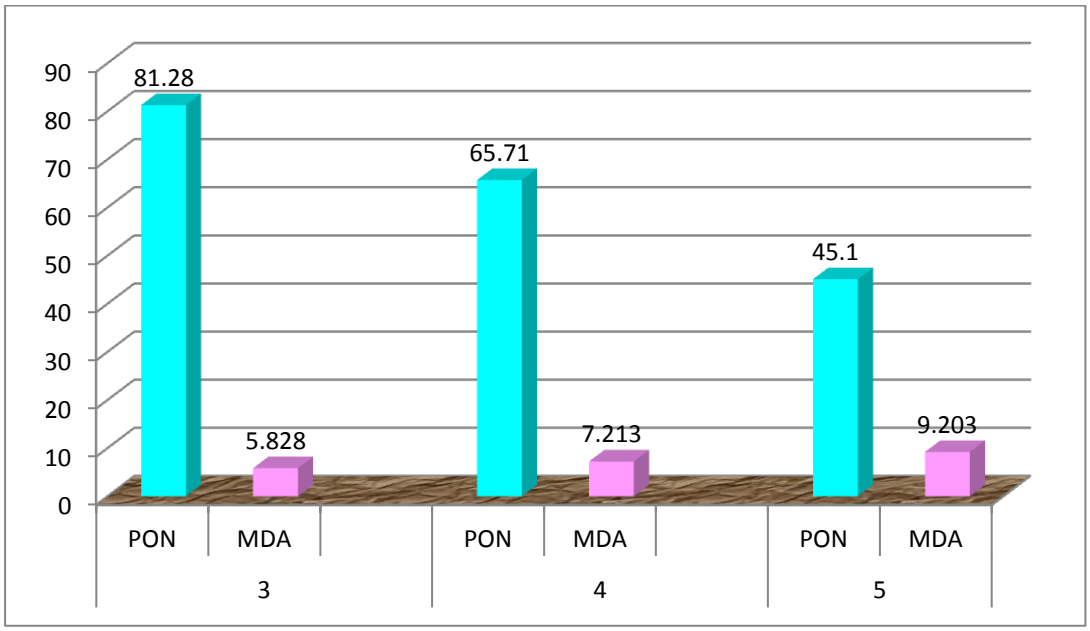
TABLE 21

MEAN PARAOXONASE& MDA LEVELS STRATIFIED BY THE NUMBER OF COMPONENTS OF METABOLIC SYNDROME

MEAN PON-1	NO OF PERSONS SATISFYING CRITERIA	NO OF CRITERIA SATISFIED	MEAN MDA
81.28	13	3	5.828
65.71	12	4	6.153
45.10	25	5	9.203

BARCHART 11

MEAN PARAOXONASE& MDA LEVELS STRATIFIED BY THE NUMBER OF COMPONENTS OF METABOLIC SYNDROME



RESULTS AND STATISTICAL ANALYSIS

A total of 100 subjects were included in the present study. Out of this 100, 50 were under the study group (Metabolic Syndrome) and the other 50 were under the control group (healthy individuals).25 Male and 25 female subjects in control and study group in the age group of 21-60 years were included in this study.

The serum PON1 activity and the levels of Malondialdehyde ,blood glucose , total cholesterol, triglycerides, HDL, blood urea and serum creatinine were estimated, for all the samples collected for the study. VLDL and LDL values were calculated Anthropometric Measurements & BP were measured & BMI were calculated .The values obtained in both the control and study groups are presented in the master chart I and II.

Table 1 shows descriptive statistics of control& study groups which include the mean values of Age, Anthropometric Data,BP,Serum PON,MDA, Blood Sugars &Lipid Parameters.

Table 2 shows Age & Sex distribution in Study & Control groups.40% of the Control group is between 21-40 yrs of age.38% of the Study group is between 21-40 yrs of age. 60% of the Control group is between 41-60 yrs of age. 62% of the Study group is between 41-60 yrs of age.

Table 3 shows that mean serum PON1 activity in Study group (59.32 ±19 U/L) is lower than the control group (154.84 ±30.71 U/L). There is a highly significant difference between the two groups.(p=<0.0001).

Table 4 shows that mean Serum Malondialdehyde values in study group (7.81 ± 3.8 µmol/L) is significantly increased, than that of control group (2.809±1.40). (P= <0.0001).

Table 5

Student's t- Test analysis of BMI between Control & Study group.

In this table there is statistical elevation of mean BMI level in study group of (32.68 ± 1.88) when compared to mean BMI level in control group of .(23.65 ± 3.58) which is statistically significant.

Table 6

Student's t- Test analysis of Waist Circumference between Control & Study group.

In this table there is statistical elevation of mean Waist Circumference level in study group of (110.26 ± 10.77) when compared to mean Waist Circumference level in control group of (89.1 ± 7.62) which is statistically significant.

Table 7

Student's t- Test analysis of Fasting Blood Glucose between Control & Study group.

In this table there is statistical elevation of mean Fasting Blood Glucose level in study group of (107.36 ±8.80) when compared to mean Fasting Blood Glucose level in control group of (91.68±6.00) which is statistically significant.

Table 8

Student's t- Test analysis of Systolic & Diastolic BP between Control & Study group.

In this table, there is statistical elevation of mean Systolic BP(133.18 ±7.575&,Diastolic BP(84.76 ±4.118) of study group ,with the mean values of Systolic BP(125.84 ±6.65),Diastolic BP(80.14 ± 2.40) of control group .

Table 9

Student's t- Test analysis of Triglycerides between Control & Study group.

In this table there is statistical elevation of mean Triglycerides level in study group of (161.54± 22.11) when compared to mean Triglycerides level in control group of (115.12 ± 18.02) which is statistically significant.

Table 10

Student's t- Test analysis of lipid profile between Control & Study group.

This table shows statistical elevation between mean values of Total cholesterol (198.64 ± 20.63), Triglycerides (161.54± 22.11), LDL (130.95 ± 20.44) and VLDL (32.30 ± 4.42) of the study group with the mean values of total cholesterol (150.54 ± 19.77),TGL (115.12 ± 18.02), LDL (79.156 ± 19.59) and VLDL (23.024 ± 3.6) of the control group showed a significant increase of all the above parameters in the study group. (P= <0.0001) .

Table11 Paraoxonase-1 activity between 3, 4 & 5 components of Metabolic Syndrome is statistically significant.

Table 12

Pearsons correlation between Serum Paraoxonase-1 activity & other Parameter.

This Table shows PON has positive correlation with HDL which is statistically significant, Negative correlation with Age, MDA, BP, WC, Weight, BMI, Blood sugar, T.Cholesterol, TGL,VLDL & LDL which is statistically significant.

Table 13

Pearsons correlation between Serum MDA level & other Parameters.

This Table shows MDA has positive correlation with Age, BP, WC, weight, BMI, Blood sugar, T.Cholesterol, TGL,VLDL & LDL which is statistically significant, Negative correlation with PON & HDL which is statistically significant.

Table 14

Pearsons correlation between Serum BMI level & other Parameters.

This Table shows BMI has positive correlation with Age, BP, WC, weight, Blood sugar, T.Cholesterol, TGL, VLDL & LDL which is statistically significant, Negative correlation with PON & HDL which is statistically significant.

Table 15

Pearsons correlation between Serum TGL level & other Parameters.

This Table shows TGL has positive correlation with Age, BP, WC, weight, BMI, Blood sugar, T.Cholesterol,VLDL & LDL which is statistically significant, Negative correlation with PON & HDL which is statistically significant.

Table 16

Pearsons correlation between HDL & other Parameters.

This Table shows HDL has positive correlation with PON-1 Activity which is statistically significant,Negative correlation with Age,MDA, BP, WC,Weight, BMI, Blood sugar, T.Cholesterol, TGL,VLDL & LDL which is statistically significant.

Table 17

Pearsons correlation between Waist Circumference level & other Parameters. This Table shows Waist Circumference has positive correlation with Age, BP, weight, BMI, Blood sugar, T.Cholesterol, TGL,VLDL & LDL which is statistically significant, Negative correlation with PON & HDL which is statistically significant.

Table 18

Pearsons correlation between Serum FBS level & other Parameters.

This Table shows MDA has positive correlation with Age, BP, WC, weight, BMI, T.Cholesterol,TGL,VLDL&LDL which is statistically significant, Negative correlation with PON & HDL which is statistically significant.

Table 19

Pearsons correlation between Systolic BP& other Parameters.

This Table shows Systolic BP has positive correlation with Age, WC, weight, BMI, Blood sugar, T.Cholesterol, TGL,VLDL & LDL which is statistically significant, Negative correlation with PON & HDL which is statistically significant.

Table 20

Pearsons correlation between Diastolic Bp& other Parameters.

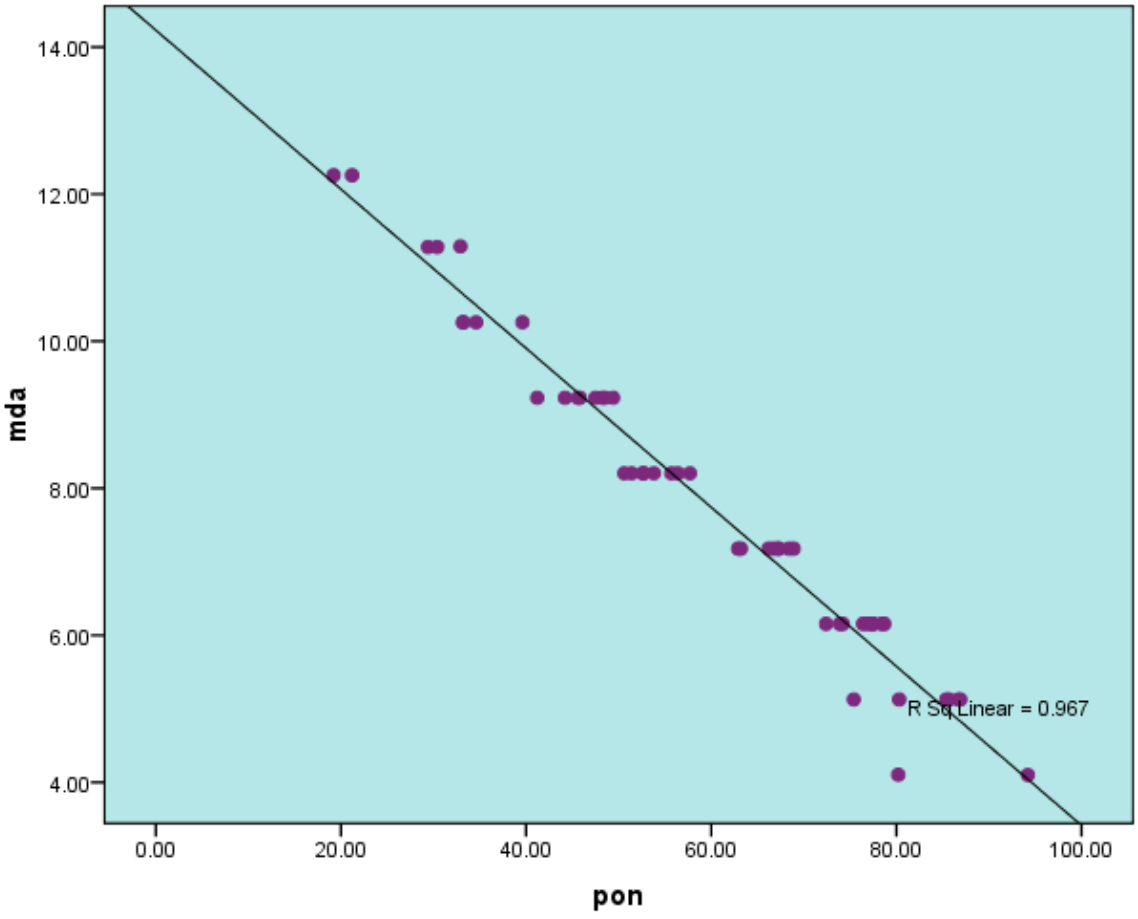
This Table shows MDA has positive correlation with Age, WC, weight, BMI, Blood sugar, T.Cholesterol, TGL,VLDL & LDL which is statistically significant, Negative correlation with PON & HDL which is statistically significant.

Table 21

This table shows mean PON1 activity level and Malondialdehyde level stratified by the no of components of metabolic syndrome.

- At the Mean PON level of 81.28 U/L & Mean Malondialdehyde level of 5.22 µmol/L –three components of metabolic syndrome satisfied.
- At the Mean PON level of 65.71U/L & Mean Malondialdehyde level of 6.15 µmol/L –Four components of Metabolic syndrome satisfied.
- At the Mean PON level of 45.10 U/L & Mean Malondialdehyde level of 9.203 µmol/L –three components of Metabolic syndrome satisfied.

Scatter plot Diagram to show correlation between PON-1 activity &Malondialdehyde level. As the PON 1 activity level decreases MDA levels increases.



DISCUSSION

The incidence and impact of the Metabolic Syndrome have risen to alarming proportions and there is a great need for therapeutic and preventive measures against this major health problem. Atherosclerotic macrovascular complication (especially coronary artery disease) is the leading cause of morbidity and mortality in Metabolic syndrome.[101]

Dyslipidemia in Metabolic Syndrome constitutes an important determinant of atherosclerosis.[102] HDL is one of the important, independant protective factor for atherosclerosis. Paraoxonase-1(PON-1) is an enzyme that confers the antiatherogenic and antioxidant properties to HDL. PON-1 is an HDL associated antioxidant enzyme, which diminishes the LDL oxidation and prevents the proinflammatory response elicited by oxidised LDL, by hydrolysing the lipid peroxides.[103,104] Moreover PON-1 is critical, for preventing the oxidation of HDL, allowing it to maintain its function. Thus PON-1 activity is responsible for the antiatherogenic property of HDL.[105]

Reduction in PON-1 activity occurs in Metabolic Syndrome and it may be due to,

1. Increased endogenous circulating inhibitors like lipid peroxidation products.

2. Increased TNF-α in Mets leads to decreased ATP Binding Cassette Protein and decreases ABC-G1.it also decreases Apo-A1 & Apo-A1V expression all these decline in HDL levels leads to decreased PON-1 activity.
3. Increased leptin levels in Mets leads to increased generation of free radicals and it directly inhibit Paraoxonase activity.
4. Increased cytokines and acute phase proteins in Mets causes reduced PON-1 enzyme activity.

By measuring the PON-1 activity in Metabolic Syndrome, we can predict the atherosclerosis earlier, and it may help for taking early preventive measures against cardiovascular disease.[106]

The present study done with 100 subjects (50 were Metabolic Syndrome individuals and 50 were healthy subjects) supports the fact that the measurement of PON-1 activity is useful in predicting the risk of cardiovascular disease in Metabolic Syndrome.

Comparison of mean value of the serum PON-1 activity in the study group (59.32 ±19 U/L) with that of the control group (154.84 ±30.71 U/L) showed a significant fall in the study group (P= <0.0001). This study also supports the fact that there is a significant decrease in PON-1 activity in Metabolic Syndrome [85,88.]

Analysis of Serum Malondialdehyde shows, that the mean Malondialdehyde values of study group (7.81 ± 3.8 µmol/L) is significantly increased, when compared with that of control group (2.809±1.40). (P= <0.0001),thus it shows increased lipid peroxidation in Metabolic Syndrome .so,by measuring lipid peroxidation levels precautions can be taken by antioxidants to prevent atherosclerosis.

Analysis of Fasting Blood Glucose shows, that the mean values of FBS in study group (107.36 ±8.80) is significantly increased, than that of control group (91.68±6.00). (P=<0.0001) satisfies one of the component of Metabolic Syndrome.

Analysis of parameters of lipid profile shows, that the mean values of HDL in study group (35.38 ±4.60) is significantly decreased, than that of control group (48.36 ±4.73). (P= <0.001).

Comparison of mean values of Total cholesterol (198.64 ± 20.63), Triglycerides (161.54± 22.11), LDL (130.95 ± 20.44) and VLDL (32.30 ± 4.42) of the study group, with the mean values of total cholesterol (150.54 ± 19.77), TGL (115.12 ± 18.02), LDL (79.156 ± 19.59) and VLDL (23.024 ± 3.6) of the control group showed a significant increase of all the above parameters in the study group. (P= <0.0001) The difference in the level of

these parameters, between the two groups are associated with dyslipidemic changes, which are the components of Metabolic Syndrome .

Comparison of Mean Values of Waist circumference (110.26±10.77cms),Weight(88.22±8.23kgs),BMI(32.68±1.88),Systolic BP(133.18±7.575) ,Diastolic BP(84.76±4.118)of study group ,with the mean values of Waist circumference (89.1±7.62cms), Weight (62.64±10.88kgs),BMI(23.65± 3.58),Systolic BP(125.84±6.65) ,Diastolic BP(80.14 ±2.40) of control group showed a significant increase of all the above parameters in the study group,(P= <0.0001).Thus this above values satisfies the components of Metabolic Syndrome.

In the Pearson's correlation analysis, the PON activity shows highly significant negative correlation with Age, BP, WC, BMI, FBS, and TGL.P(<0.001).It shows that PON Activity decreases with the components of metabolic syndrome. Age is the non negligible factor, other than Age, remaining could be modifiable with appropriate Exercise, Life Style changes,& drugs in order to prevent atherosclerosis.

The levels of HDL shows a significant positive correlation with the PON activity.P= <0.001 & negative correlation with Malondialdehyde, Age, BP, WC, BMI, FBS, and TGL.P (<0.001) this correlation proves the association of PON with HDL.

In the Pearson's correlation analysis, Serum Malondialdehyde levels shows highly significant Positive correlation with Age, BP, WC, BMI, FBS, and TGL.P(<0.001).It shows that Malondialdehyde levels increases with the components of metabolic syndrome. Age is the non negligible factor, other than Age, remaining could be modifiable with appropriate Exercise, Life Style changes,& drugs in order to prevent Oxidative stress and other events related to oxidative stress .

At the Mean PON level of 81.28 U/L & Mean Malondialdehyde level of 5.22 µmol/L –three components of Metabolic syndrome are satisfied. At the Mean PON level of 65.71U/L & Mean Malondialdehyde level of 6.15 µmol/L –Four components of Metabolic syndrome are satisfied. At the Mean PON level of 45.10 U/L & Mean Malondialdehyde level of 9.203 µmol/L –three components of Metabolic syndrome are satisfied.

As the number of components of Metabolic Syndrome increases mean PON 1 Activity decreases & mean MDA levels increases.Thus it shows increases in Lipid Peroxidation with decreased in PON activity.

CONCLUSION

This study shows that there is a significant decrease in PON-1 activity in Metabolic Syndrome group and there is significant increase in Malondialdehyde .Since PON-1 is an antiatherogenic and antioxidant enzyme, associated with HDL, reduction in PON-1 activity in Metabolic Syndrome, may play an important role in causation of premature atherosclerosis.[107,108]

Based on the results obtained the present study shows that PON-1 activity may be used as a marker for early prediction of atherosclerosis in Metabolic Syndrome. By predicting earlier, early interventional measures by pharmaceutical means or by dietary means can be done.[69,73,98,99]

LIMITATIONS OF THE STUDY

1) HOMA-IR index could have been measured for these individuals.

2) Studies on gene polymorphism would have helped to evaluate the discordance in human PON-1 gene between phenotypes and genotypes in Metabolic Syndrome.

SCOPE FOR FURTHER STUDY

The intake of nutritional antioxidants such as carotenoids (lycopene and beta carotene), vitamin C, and polyphenols (in tea, red wine, grapes, licourice root, and pomegranate) by atherosclerotic animals, leads to reduction of oxidative stress and to the attenuation of atherosclerosis development. This could be related to increase in HDL –PON -1 activity through effects on gene expression, or preventing enzyme inactivation and on increasing PON-1 stability through it's binding to HDL.[65,69,73,109, 110]

Studies on dietary determinants of serum PON activity in human beings can be done in future, so that timely intervention can be done to prevent atherosclerosis.

It has been discovered recently that orally administered short peptide sequences resembling the amphipathic helices of Apo A-1, when synthesized from D-amino acids (to avoid gastric proteolysis) will be active in preventing atherosclerosis in animal models. One of these D-4F administered orally to apo–E null mice, decreased atherosclerosis and also decreased circulating lipid hydro peroxides and increased pre –β HDL and PON activity.Addition of D-4F directly to human plasma also decreased lipid peroxides and increased the PON activity .[111]

In future, to increase the antioxidative activity of HDL, an approach to increase the concentration of selective HDL component such as PON -1 by somatic gene transfer may prove beneficial.[111]

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A STUDY OF SERUM PARAOXONASE-1 ACTIVITY IN PATIENTS WITH METABOLIC SYNDROME

PROFORMA

NAME OF THE PATIENT :

AGE :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

GENERAL EXAMINATION:

Ht: Wt: BMI: BP: PR:

SYSTEMIC EXAMINATION:

CVS: RS:

ABD: CNS:

INVESTIGATIONS :

1. BLOOD SUGAR : FBS: PPBS:

2. SERUM PARAOXONASE:

3. SERUM MAlONDIALDEHYDE

4. BLOOD UREA:

5.SERUM CREATININE:

6.LIPID PROFILE:

TOTAL CHOLESTEROL:

TRIGLYCERIDES:

HDL:

LDL:

VLDL:

CONSENT FORM

Dr.T.Rajalakshmi post graduate student in the department of Biochemistry, Thanjavur medical college, Thanjavur is doing a Study on Serum Paraoxonase -1 Activity in Patients with Metabolic Syndrome. The procedure has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature :

Name:

Place: